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Columbia University
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College of Physicians & Surgeons, Dept. of Surgery

Studies from the Laboratories of the Department of Surgery

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Volume II, 1910-1917

PREFACE

The laboratory of Surgical Research of the College of Physicians and Surgeons was organized at the suggestion of Dr. William T. Bull in 1903. At that time the facilities were few and the scope in research was necessarily narrow.

From 1903 to 1913 while Dr. Joseph A. Blake was professor of surgery, many improvements were instituted in the construction and equipment of laboratories for the department of Surgery.

About 1905 the need was felt for a laboratory of Surgical Pathology in which studies upon the tissues could be made. To fill this want a desk was provided for a time in the Laboratory of Pathology. On Lincoln's birthday, 1910, a Laboratory of Surgical Pathology was opened, and in 1911 a new and spacious laboratory devoted to Surgical Research was completed. Much time has been required to organize and to develop the work of these two laboratories, now a unit in the Department of Surgery.

At first, chiefly methods of technique were studied. With the addition of the laboratory of Surgical Pathology and the cooperation of its staff, studies in microscopical morphology were commenced. As a result, research workers have the opportunity of confirming with the aid of the microscope many of their gross observations of structural and functional changes which are the result of experiment. In this way more accurate observations are possible and more careful work results. There are conducted also, studies of the tissues in inflammation, in injury and infection; similarly the many and varied tumors are examined and classified always with the one end in view of increasing the scope of investigation. Particularly, research through the use of an incubator in which tissue cultures have been grown, has widened the fields of investigation in surgery.

In order to make real progress in the study of surgery, knowledge of medicine is required, as well as a knowledge of biology, of chemistry and of physics. On account of this extension in the science of surgery, workers in these several branches are necessary and some of them at present are on the laboratory staff. With these increases in personnel, there is a constant demand for additional apparatus and the necessary room for its installation.

In 1907, the first volume of the published studies from the department was issued. This second volume of reprints represents the published research, experimental and other work since 1911, when the two laboratories were combined under one head. These investigations have been carried on in widely scattered fields and serve to emphasize the scope in the science of surgery.

A perusal of the papers in this volume will occasionally disclose opposed viewpoints and conclusions. It is the policy of the laboratory to encourage individuality and each publication represents the personal views of the writers. It is clear that perfect agreement cannot be attained because finality can never be reached in science. While a certain responsibility is assumed for accuracy in reports of experiments upon which the publications are based, none is assumed when conclusions are drawn without the aid of the microscope or other instruments of precision.

Signed

GEORGE E. BREWER

TABLE OF CONTENTS

1. The Development of the Blastoderm of the Chick in Vitro. John E. McWhorter and Allen O. Whipple.

2. An Apparatus for Intratracheal Insufflation. Henry H. Janeway.

3. Adaptation of the Cinematograph to the Study of Embryology and Tissue Growth. John English McWhorter and Frederick Prime.

4. The Possibilities of Preserving the Integrity of Potential Body Cavities by the Use of a Foreign Body to Prevent Adhesions. Frederick Prime, Jr.

5. The Function of the Periosteum in Bone Transplants, Based on Four Human Transplantations without Periosteum and some Animal Experiments. Clarence A. McWilliams.

6. The Periosteum in Bone Transplantations. Clarence A. McWilliams.

7. The Use of Herudin in the Transfusion of Blood. Henry S. Satterlee and Ransom S. Hooker.

8. The Process of Bone Repair Following Trauma. Frederic W. Bancroft.

9. A Course in Surgical Technic. William Cogswell Clarke and Frederic W. Bancroft.

10. Reliability of Dogs as Subjects for Experimental Nephritis. Hughes Dayton.

11. On the Lymphatic Drainage of the Peritoneal Sac. William Cavan Woolsey.

12. Thyroid and Circulatory Changes Following Experimental Ligation of the Thyroid Vessels. Harold Neuhof.

13. Experimental Subarachnoid Injections of the Trypan Blue. William Cavan Woolsey.

14. The Anatomic Distribution of Cutaneous Neoplasms. F. T. Van Beuren, Jr.

15. Experimental Mesothelium. William Cogswell Clarke.

16. A Transplantable Chondro-Osteosarcoma in a Dog. John E. McWhorter and Frederick Prime.

17. The Inefficacy of Pyloric Exclusion by Fascial Bands. Harold Neuhof.

18. A Preliminary Report on a Simple and Rapid Method of Pyloric Closure in Gastro-Enterostomy. George Emerson Brewer.

19. Pyloric Exclusion. Richard Lewisohn.

20. The Process of Repair in Wounds of the Small Intestine. John E. McWhorter, A. P. Stout and Charles C. Lieb.

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March, 1912

THE DEVELOPMENT OF THE BLASTODERM OF THE CHICK *IN VITRO*[1]

JOHN E. McWHORTER[2] AND ALLEN O. WHIPPLE[3]

Columbia University, New York City

TWELVE FIGURES[4]

Since Harrison's publication in 1907[5] in which he described the development *in vitro* of the nerve fibers of the frog embryo, experimental work in growing tissues has been largely confined to the culture of bits of tissue removed either from the embryo or adult animal or from various tumors. The development *in vitro* of the chick embryo, so far as we are aware, has not been described.

The rhythmical and vigorous contractions, for over five days, of the heart removed from a forty-hour chick embryo and planted in plasma, suggested to us the study of the developing vascular system. A seventy-two hour embryo was planted in plasma. The heart continued to beat uninterruptedly for over nine hours. During this time the entire vascular system could be seen in action under the microscope. While observing this first specimen we were impressed with the possibilities which such a method offers for the study of various problems in embryology and pathology. We began accordingly to test the viability and developmental possibilities of younger embryos. In this work we employed, in a modified form, the apparatus and technique which we have been using during the past year in growing adult and embryonic tissues.

[1] Read before the American Association of Anatomists, December 27, 1911, at Princeton, N. J.

[2] From the Department of Surgery, College of Physicians and Surgeons, Columbia University (The George Crocker Special Research Fund).

[3] From the Department of Surgery, College of Physicians and Surgeons, Columbia University.

[4] Expense of illustrations borne by authors.

[5] Anat. Record, vol. 1, 1907.

THE APPARATUS

The modified incubator is shown in the accompanying photograph (fig. 1). Its chief advantages are: First, observation of the specimen on a mechanical stage controlled from the outside, without alterations in temperature; Secondly, the opportunity offered to take low and high power photomicrographs of all or parts of the specimen with an accurately focused, condensed, and heat filtered light.

The incubator consists of two parts: a base below, above this the incubator proper containing the microscope. The base is a rectangular sheet-iron box similar to the usual type excepting for an air space which is obtained by means of a heavy iron plate placed 7 cm. below the under surface of the incubator. The purpose of this air space is to maintain a more equable temperature within the incubator.

The incubator proper, which rests upon an iron rim on the base, consists of a wooden box thoroughly insulated on its outer surfaces with asbestos. To its lower surface, is attached a perforated iron plate; the perforations are continuous with similar ones in the asbestos and wooden floor. These warm air inlets are plugged with glass tubes containing glass wool for filtering the air. Through the upper surface projects the draw tube of the microscope and immediately behind this is a small aperture for the extension rod connecting with the fine adjustment; to the right is a small door giving access to the incubator. A window in front transmits light to the mirror of the microscope. Above and to the side of this window is an opening for the controlling device of the Bean's heat regulator. At the back are two small openings, one above the other, for cords attached to the lever of the iris diaphragm. On the right side are extension rods controlling the coarse adjustment of the microscope and the two controls of the mechanical stage. On the left side is a door of ample size to allow free access to the incubator. Back of this passes an extension rod to control the substage. One arm of a right angled thermometer passes through this side and is so situated that its bulb lies immediately below stage of microscope. The tempera-

ture of the incubator is maintained by means of the small Bunsen burner in the base. The Bean's heat regulator as a thermostat has proved quite satisfactory as the temperature within the incubator seldom shows a variation of more than one degree.

The device here used for photomicrography consists, as is shown in the photograph, of a camera attached by means of a thumb screw to a slotted plate. This plate is kept rigid by being screwed to a frame work of iron piping, which in turn is securely fastened to the floor and braced. When taking photographs the camera is lowered to the lower limit of the slotted plate and the brass tube on the lens board of camera is inserted into the sleeve of the collar surrounding the draw tube of the microscope. When not in use the camera is disconnected from the microscope and pushed to the upper limit of the plate and clamped.

The method used for illuminating the field for photography is similar to that generally used in photomicrography, the only difference being that in this work the use of a heat filter is essential, for the concentrated heat rays from the small arc lamp, if not filtered, cause tissue death in a very few seconds.

THE TECHNIQUE

Fresh eggs are incubated at 37° to 39° C. Using aseptic precautions throughout, the blastoderm is removed from the egg by cutting wide of the area vitellina externa and lifting it out of the yolk with sufficient adherent yolk to prevent injury. The blastoderm is transferred to Locke's solution, kept at 37° C. on a water bath. Yolk granules and vitelline membrane are removed by gently squirting the solution against the blastoderm with a medicine dropper. The blastoderm is then floated onto a cover glass with its dorsal or upper surface in contact with the cover glass; the excess of Locke's solution is removed with sterile absorbent cotton. A few drops of plasma are placed on the blastoderm and when this has coagulated the cover glass is inverted over a hollow glass slide, containing a drop of water, and rimmed with paraffine. The specimen is then incubated at 38° C.

Plasma is obtained from the adult fowl by the method described by Carrel and Burrows, that is, blood is drawn from the external jugular vein through a glass canula, coated with olive oil, into ice cold paraffine tubes. The blood is centrifuged and the plasma is then refrigerated. We have used plasma either pure or mixed in different proportions with Locke's solution. When used unadulterated the specimen is securely fixed to the cover glass. This obviates the sagging and blurring of the specimen. The disadvantage in its use is the mechanical resistance which the jelly-like film, with its fibrin network, necessarily offers to the free expansion of the embryo in its growth. By means of special glass slides in which the blastoderm rests on a depressed disc in a mixture of Locke's solution and plasma or serum the hanging drop method is avoided. We hope to get better results by this means, which we have only recently employed.

The chief factors in preventing prolonged growth in our series of blastoderms have been:

1. Injury: from rough handling, drying or cooling.
2. The limited supply of oxygen and nutriment. This difficulty we expect to overcome, in part at least, by improvements in the chamber.
3. The prolonged or too frequently repeated exposure of the blastoderm to the strong light in making photomicrographs. The plasma in many such cases liquefied much more rapidly than usual.

OBSERVATIONS

Our attempts to grow embryos before the appearance of the head fold have been unsuccessful, largely because of the difficulty of removing the blastoderm from the egg at such an early stage. But from the 3 – 4 somite stage up to 17 – 18 somites we have been able to watch continuous development. Embryos removed after the beginning of the heart beat, the 10 – 12 somite stage, have lived a variable length of time, the longest thirty-one hours. Comparing our specimens that have grown, as determined by the increase in somites and development of the nervous and vascular systems with those removed from the egg at corresponding stages

of incubation it is evident that development *in vitro* is practically the same as *in ovo*.

By this method continuous observations, hitherto impossible, can be made in the development of the primary divisions of the brain, the optic and the otic vesicles, the relation of the folds of the amnion to the splanchnopleure and somatopleure, the folding of the heart, with the cephalic progression of the auricles, and the process of somitic division. It is in the field of angiogenesis, however, that we believe the study of the blastoderm *in vitro* offers special opportunities.

We wish to make a preliminary report on observations made in the blastoderm before the establishment of well formed blood vessels.

In the area pellucida, with the embryo at the 3–6 somite stage, spaces appear, first at the margin of area opaca, shortly afterward in area pellucida and margin of embryonic body wall. The best place to observe them is in the area pellucida. Here they appear at first as isolated spaces, of various shapes and sizes, in an undifferentiated layer of mesenchymal cells beneath the ectoderm (fig. 7, *1*, *2*, *5*). These spaces are frequently bounded by a mere line, more or less refractile in character (fig. 7, *1* and 2). In others the lumen is lined with rounded or oval cells which later become fusiform and flattened (fig. 8, *2* and *4*). In not all of these spaces is there a well defined limiting wall. Many of them end blindly in undifferentiated mesenchyme (fig. 7, *1* and *5*).

Under observation, these isolated spaces have been seen to change their shape, to expand, and to unite with similar spaces figs. 9, 10). In some specimens this union of isolated spaces is quite a rapid one and, unless observed during a limited period the process cannot be seen. After the 10 somite stage we have been unable to detect isolated spaces. With the confluence of spaces channels are formed (fig. 11). At times they show a fairly well defined lining of flattened cells. Under the strain of increasing fluid content these channels, situated as they are in the soft ooze of mesenchyme, dilate and a bulging of their walls occurs in their weaker parts. Where the bays, or recesses, of adjacent channels meet a communication is established and a plexus is

formed. This plexus formation is first noted at the level of the anlage of the omphalo-mesenteric veins (fig. 4, *2*). With the establishment of the heart beat plexus formation progresses rapidly. A remarkable example of the effect of the hydro-dynamics of the pumped fluid in these channels was seen in No. 36 of our series. After the heart had been beating steadily for five hours the plasma covering the blastoderm liquefied and the embryo sagged, while the area opaca remained adherent to the cover glass. This caused a kinking of the left omphalo-mesenteric vein. Immediately many of the channels in the area pellucida tributary to the kinked vein became engorged and the process of bulging of the walls in their weaker parts, with the formation of new channels became strikingly apparent. In two instances the walls of parallel channels were pushed together. In one case, after the walls had been in contact a half hour a new opening formed and blood cells rushed from the vessel of greater blockage into the more rapid stream of the other vessel. The same specimen showed vessels which had collapsed, owing to the shifting of the blood stream to other channels. Within an hour the cells lining these channels had lost their endothelial character and reverted to the undifferentiated type of mesenchymal cell.

The presence of a fluid in these channels was well demonstrated in several of our specimens. For prior to the establishment of the circulation isolated cells or groups of cells were seen changing their position in the lumen of the channels. With the onset of the heart beat there began a to and fro motion of these cells. Finally, as the circulation became established in a portion of the plexus these cells, or groups of cells breaking off from cell masses, suddenly shot through the channels as if an obstruction was suddenly removed. In the plexus, in the neighborhood of the omphalo-mesenteric veins, the direction of travel of these first cells was always from the area opaca to the sinus venosus. In one of our series (figs. 5 and 6) we were able to observe the development of the plexus in this region, the appearance of the heart beat and of the first two folds of the heart, the early oscillating movements of blood cells with their later streaming toward the

sinus venosus, and finally the passage of blood cells from the area opaca, through the plexus, to the sinus venosus, through the heart, down the left dorsal aorta, out of the vitelline artery, back to the area opaca.

The phenomena of the development of isolated spaces in the area pellucida; their confluence, resulting in channels; the elaboration of a plexus from these channels; the effect of the hydrodynamics of fluid, pumped by the heart through these plexuses, in establishing well defined blood vessels have been observed by us in our blastoderms. In some cases several of the stages have been watched in the same embryo. As yet we have been unable to follow all of the successive stages in the same specimen.

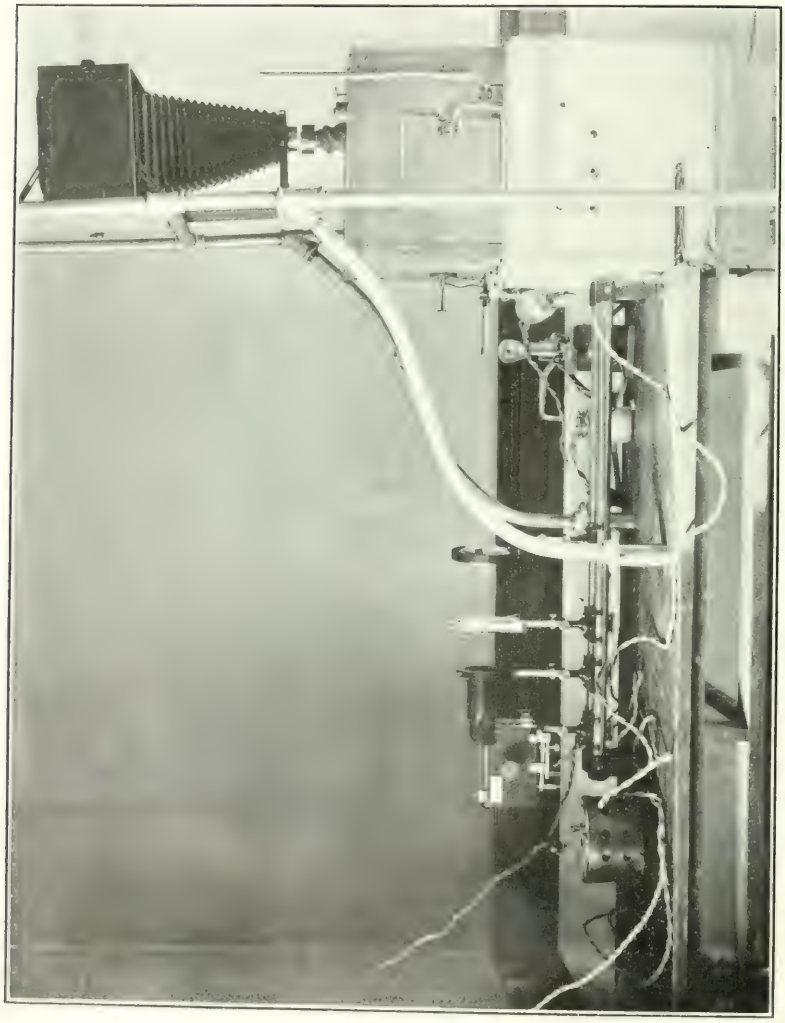

Fig. 1. Photograph of modified incubator.

Fig. 2 Photomicrograph of embryo at 6 – 7 somite stage. Note incomplete closure of neural fold. This specimen lived eighteen hours. × 28.

Fig. 3 Photomicrograph of same embryo as in fig. 2 shows 10–11 somites. Note development of fore brain. This specimen developed heart beat. × 28.

Fig. 4 Photomicrograph of embryo showing amnio-cardiac vesicles. × 28.
1 1.

Fig. 5 Photomicrograph of embryo showing 12 – 13 somites. Heart and partial circulation developed under observation. × 28.

Fig. 6 Photomicrograph of same embryo 14 – 15 somites with further folding of heart. × 28.

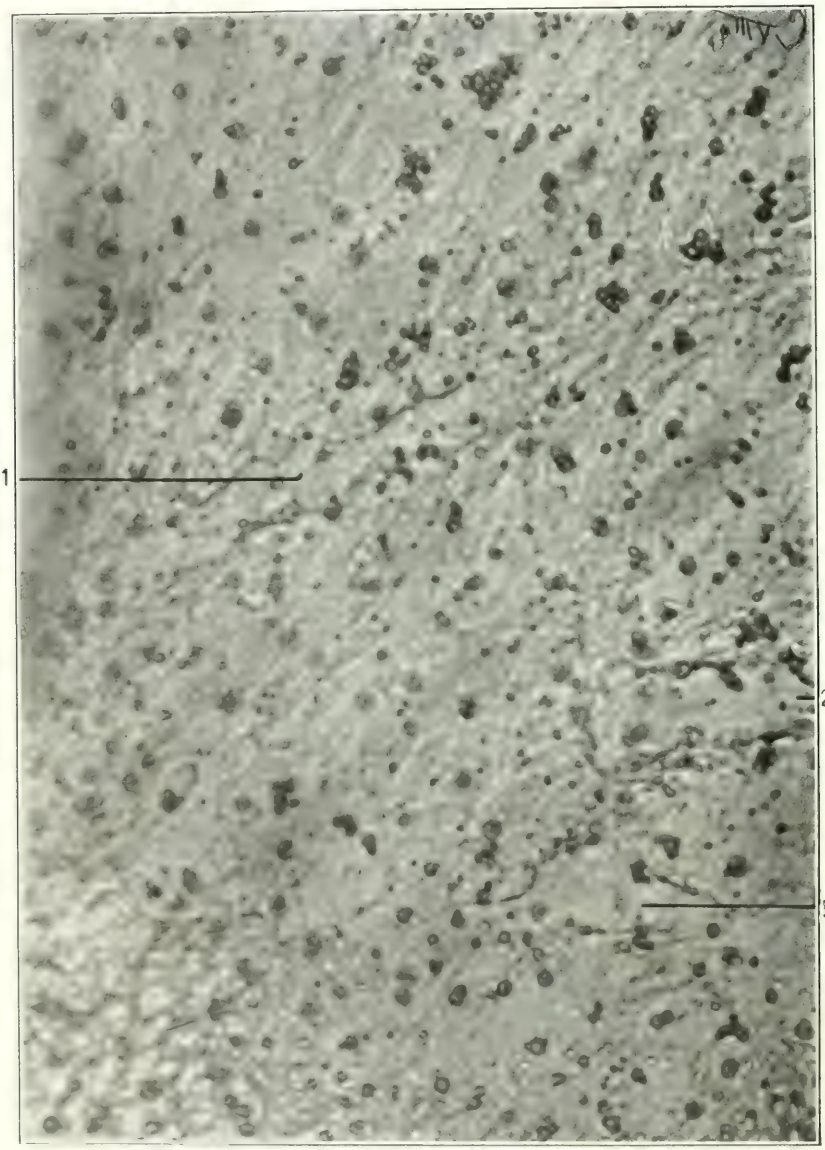

Fig. 7. Photomicrograph of isolated spaces *1* - *2* - *5*. These walls are refractile showing few cells. Note merging of space *5* in undifferentiated mesenchymal cells. × 500.

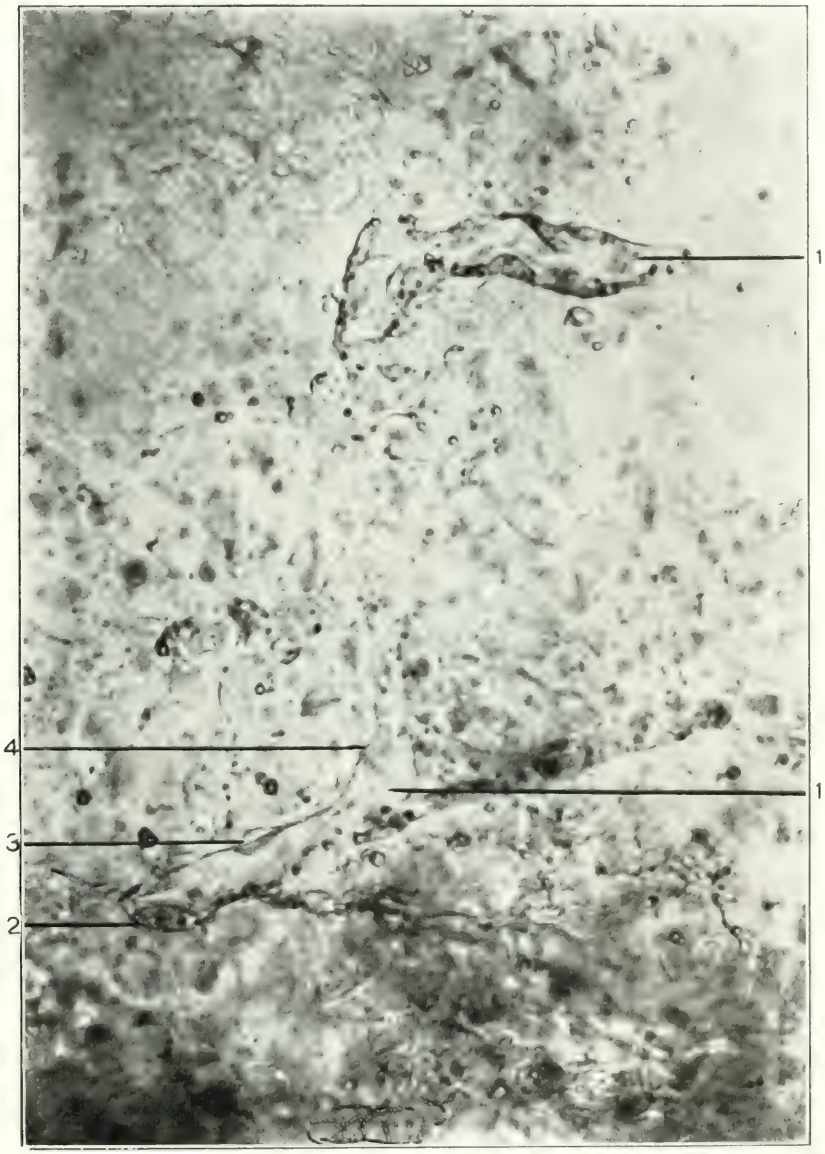

Fig. 8 Photomicrograph of two isolated spaces *1 – 1*. Note various shapes of cells *2 – 3 – 4* lining the spaces. × 500.

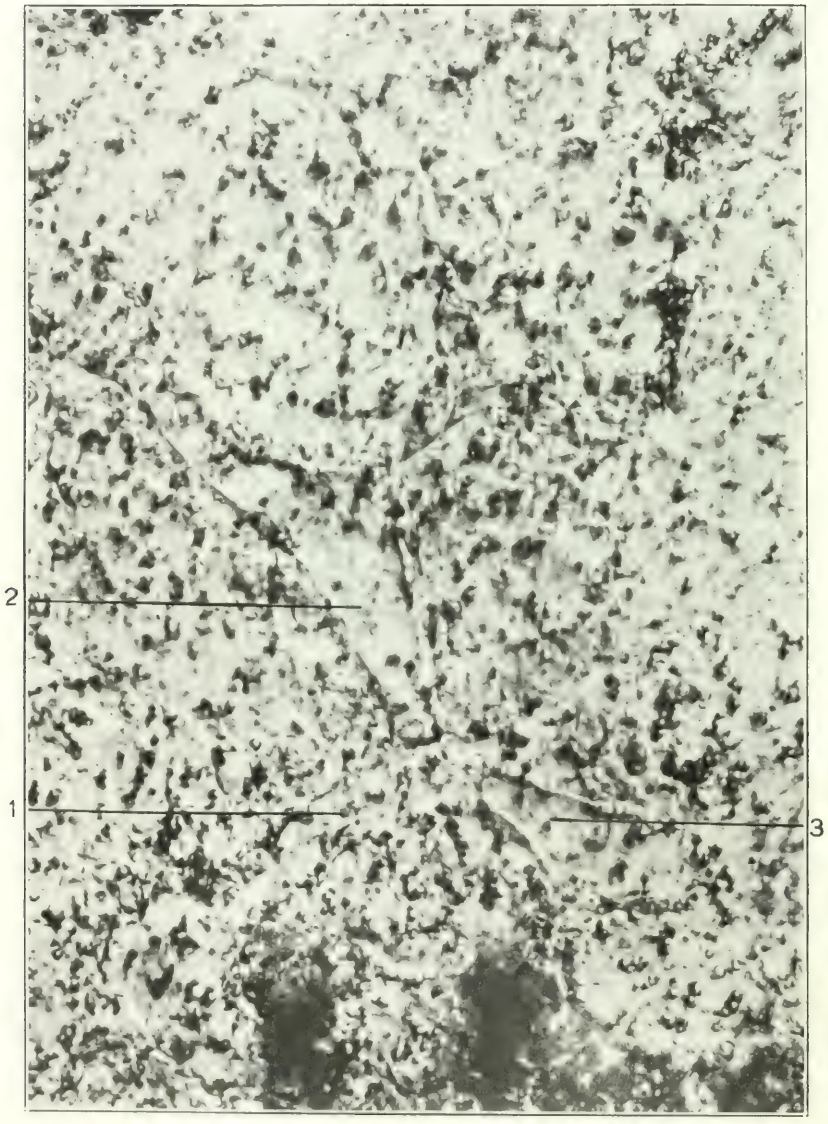

Fig. 9 Photomicrograph of isolated spaces *1 – 2 – 3*, about to unite. × 500.

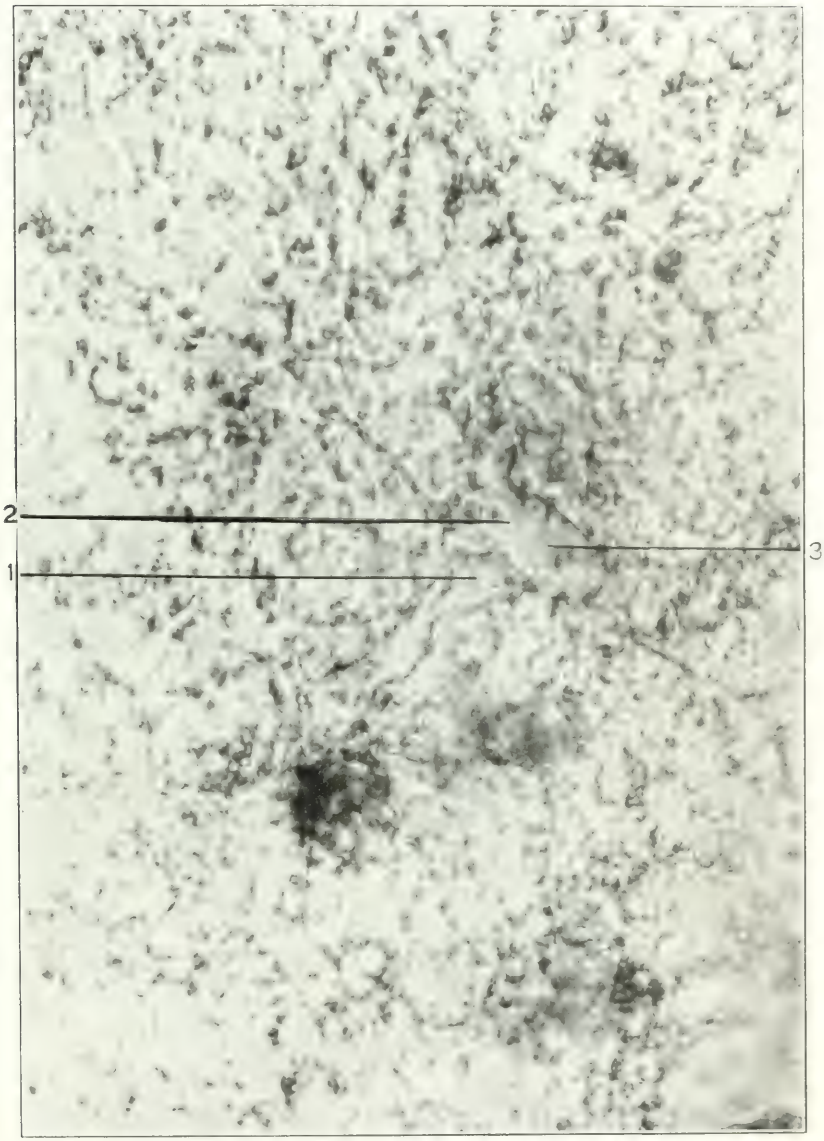

Fig. 10 Photomicrograph of same spaces *1 – 2 – 3*, as shown in fig. 9 now united. × 500.

Fig. 11 Photomicrograph of channels. ×500. 1.

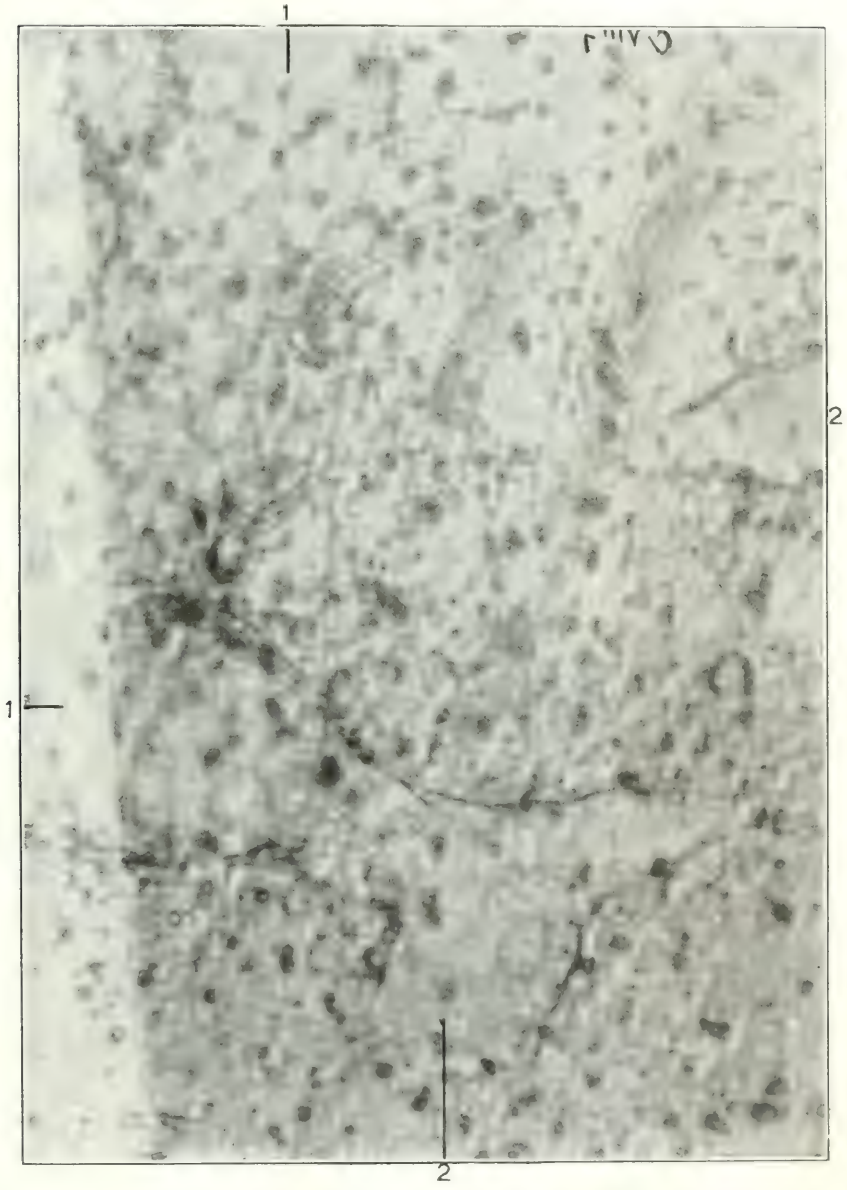

Fig. 12 Photomicrograph of channels *1 – 1* with evaginations *2 – 2* going on to plexus formation. × 500.

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AUGUST, 1912

AN APPARATUS FOR INTRATRACHEAL INSUFFLATION.

(From the Laboratory for Surgical Research, Columbia University.)

BY HENRY H. JANEWAY, M.D.,
OF NEW YORK CITY.

THE accompanying photographs represent an apparatus for the administration of anæsthesia by intratracheal insufflation. It embodies a number of ideas applied to intratracheal insufflation which have been the outgrowth of the previous work of Dr. Nathan Green and the writer in experimental intrathoracic surgery.

The apparatus furnishes to the patient a continuous current of warmed, moistened, and filtered air, mixed with any desired amount of ether vapor, and interrupted at regular intervals, in order to allow at such intervals of partial collapse of the lungs. At the present time the advantages of such a method of anæsthetization by intratracheal insufflation are abundantly substantiated. The apparatus is easily portable, measures only 18″ × 8″ and will run upon both the alternating and direct current.

The current of air is supplied by the rotary blower "1." This blower is of the simplest type and is in no way dependent upon the use of springs. From the blower the air passes through the filter "2" and from here through the valve "3" over the surface of the ether in the jar "4." After passing over the surface of the ether the current of air passes over the surface of the heated water in the jar "5." The water in this jar is kept at a constant temperature by an electric heater inside. By means of the valve "3" any proportion of the air current may be shunted directly over the heated water in jar "5," thus diminishing reciprocally the amount which passes over the surface of the ether, and in this manner the

amount of ether supplied to the patient. In case a greater quantity of ether is desired at any time, provision is also made by the valve "6" for bubbling a proportion of the air passing through the ether jar through the top layers of the ether irrespective of the level of the ether in the jar.

The current of air passing to the patient is interrupted at regular intervals (which can be varied) by the valve "7," operated in turn by the worm wheel "8." These interruptions are a very important part of intratracheal insufflation. Not only do they materially aid the diffusion of oxygen to the alveoli of the lungs, but they also bear a direct relation to character of the circulation by facilitating the return of the venous blood to the heart. A positive pressure maintained by a constantly distended lung around the great veins entering the heart impedes the venous flow to the heart, and, if high enough, can actually create a mechanically caused shock. For this reason as well as for safe-guarding against rupture of the lungs, the proper regulation of the pressure in intratracheal insufflation is very necessary. An accidental momentary increase of the pressure during intratracheal insufflation may cause rupture of the lungs and fatal pneumothorax with emphysema. With a medium sized catheter within the trachea and a free outlet of the air through the larynx it is difficult to realize how a pressure within the trachea sufficiently high to rupture the lungs could ever occur.

During such acts as coughing the intrapleural pressures often reach 80 to 116 mm. of mercury. The elastic recoil of the lungs after death has been estimated by Hutchinson at 4.23 mm.Hg. To overcome this pressure it is only necessary to supply air through the usual intratracheal catheter at a pressure of 20 mm. of Hg. At this pressure the intratracheal pressure is about 5 mm. of Hg. Even without a manometer to guide one, and much less with one, at first it had been considered safe to trust alone to the degree of distention of the lungs as a guide to the amount of air to be supplied to the patient. Experience, however, has proved this not to be so. Sudden accidental rises in the pressure

FIG. 1.

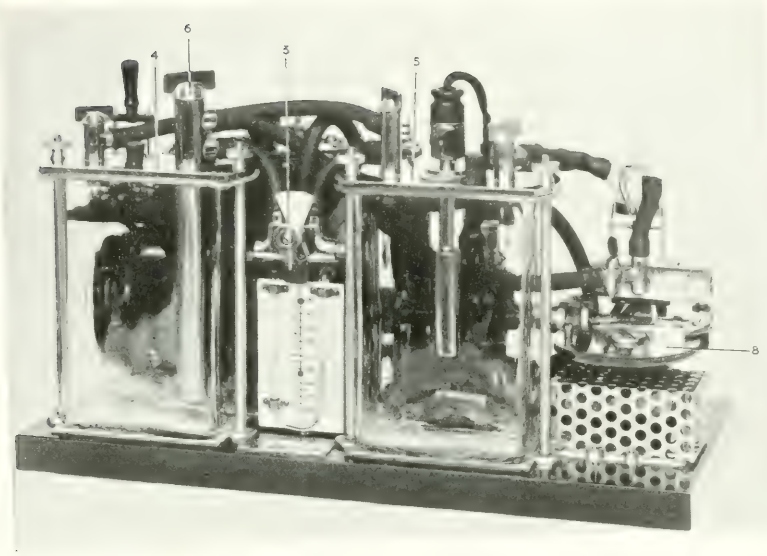


FIG. 2.

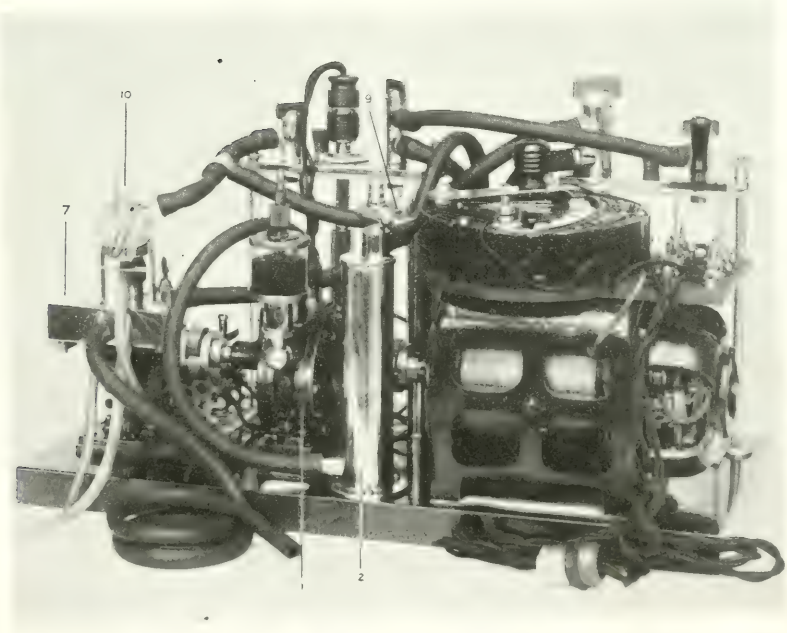


FIG. 1.—Front view. 3, valve for regulating the amount of air passed over the surface of the ether; 4, ether jar; 5, water jar; 6, valve for bubbling a portion of the air through the top layers of the ether; 8, worm wheel operating interrupting valve.

FIG. 2.—Back view. 1, blower; 2, air filter and muffler; 7, interrupting valve; 9, safety-valve controlling the pressure; 10, condensing bulb.

may occur. If the tube has not been introduced within the trachea carefully under the guide of direct vision, the latter may be so irritated that it will tightly close around the catheter, causing a sudden increase of pressure. In our own experience a death has resulted from the anæsthetist's pushing the intratracheal tube so far within the trachea that its distal end entered one bronchus. The bronchus tightly hugged the tube, with the result that all the air was blown into one lung with no way of escape. The lung was ruptured, and fatal pneumothorax and emphysema resulted. Such experiences as these demonstrate the necessity not only of carefully supervising the pressure by a manometer, but also the adoption of some means of providing automatically guarding against too great an increase of pressure.

Since the above mentioned experience we have added the blow-off valve "9" which can be adjusted to blow off at 20 or 25 mm. of Hg. The bulb "10" removes the water of condensation from the efferent current of air while the efferent tubes are becoming warmed. We have also added recently a pressure reducer to the blower which permits of the use of very small quantities of air and consequently the saving of ether without the hissing noise incidental to the blow-off valve. Its utilization is of service when ether is given by intratracheal insufflation in other cases than thoracic ones. While intratracheal insufflation may be given with a very simple apparatus depending upon a hand or foot bellows for the supply of air, we believe that the facilities and safeguards of the apparatus herein described insure greater ease and security of administration. A foot bellows should always be at hand in case of accident to the electric current. Such a bellows can be attached to the supply pipe from the blower in time of need.

ADAPTATION OF THE CINEMATOGRAPH TO THE STUDY OF EMBRYOLOGY AND TISSUE-GROWTH *

JOHN ENGLISH McWHORTER, M.D.
AND
FREDERICK PRIME, JR., M.D.
NEW YORK

Our object in taking up this work has been twofold; first, to reproduce for the benefit of students, in their study of tissue-growth and the early development of the embryo, graphic pictures of the changes that these tissues undergo; and, second, to reproduce for study such movements as are not readily followed by the eye; such, for example, as the rapidly beating embryonic heart or the exceedingly slow movements incident to the growth of tissue and cell division. From the results thus far obtained, we feel warranted in assuming that the method about to be described of adapting the cinematograph to the microscope, for the purposes of photomicrography, is at least practical and capable of much further elaboration. The embryos as well as the tissues used in these experiments were those of the chick, the pictures being taken in different stages of development.

The preparation of the chick blastoderm for the purposes of photomicrography[1] may be described briefly as follows: Fresh fertile hen's eggs are incubated for from twenty-four to sixty hours at a temperature ranging from 37.5 to 40 C. (99.5 to 104 F.). At any stage between twenty-four and sixty hours incubation, the blastoderm may be removed from the egg and planted with comparative ease. Before and after that period, it is extremely difficult, if not impossible, to remove the embryo and prepare it for mounting without inflicting sufficient injury to cause death. Assuming that the incubation has been carried on for thirty-six hours, the first step after removal from the incubator is to cut

* From the Laboratory of Surgical Pathology, Department of Surgery, College of Physicians and Surgeons, Columbia University.
* Read before the New York Academy of Medicine, Feb. 6, 1913.
1. Anat. Rec., vi, No. 3.

away a sufficient amount of shell to expose the whole upper surface of the yolk. If the egg has developed normally, we see the embryo (Fig. 1) lying on the upper surface of the yolk, surrounded by a perfectly circumscribed zone, the extra-embryonic area. The embryo, with its extra-embryonic area, is freed from the yolk with a pair of fine scissors, and with a spatula is lifted from its position and immersed in a warm isotonic solution, either Locke's or Ringer's. The embryo is freed of its adherent vitelline membrane and yolk while thus

Fig. 1. Egg, with part of shell removed, showing position of blastoderm on yolk.

immersed, floated on a thin glass cover-slip, removed from the solution and drained of any excess fluid. A few drops of chicken plasma are now allowed to flow over the specimen, which, on coagulation, attaches it smoothly and firmly to the surface of the glass. Finally, the cover-slip is inverted over a large glass cell (Fig. 2), sealed with sterile petrolatum and paraffin, and kept in the incubator at a constant temperature. In other words, we have made of our embryo a hanging-drop preparation. The preparation of tissue-cultures for this type of photography is essentially the same as that for study.

APPARATUS

As may be seen from Figure 3 the apparatus can be divided into three parts: (1) the modified incubator; (2) the optical bench; (3) the cinematograph.

The modified incubator[1] is constructed to contain a microscope on the stage of which a specimen may be kept at an equitable temperature and yet available for study. This is accomplished by projecting the draw-tube of the microscope beyond the upper surface of the box, and by means of connecting rods attached to the various adjusting screws of the

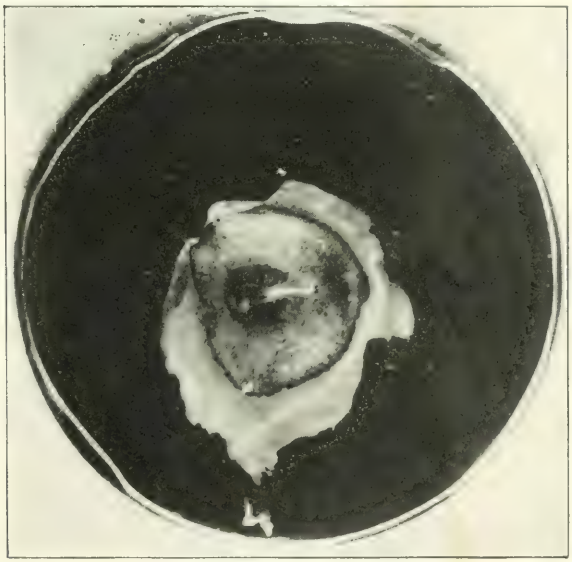


Fig. 2.—Glass cell showing blastoderm mounted and ready for microscopic study and photography.

microscope and mechanical stage. The introduction of light to this microscope is effected by transmitting the rays from the optical bench through a small window in the box to a right-angle prism immediately below the substage condenser.

The optical bench is of the usual type; namely, an arc-light, iris diaphragm condenser and glass cell. The glass cell contains water in constant circulation. Its purpose is that of a filter and it removes to a large extent the heat-rays from the condensed beam of light. The automatic self-feed arc-light of from 15 to 20 amperes is the most suitable for this work, as it maintains a more constant and even illumination of the field.

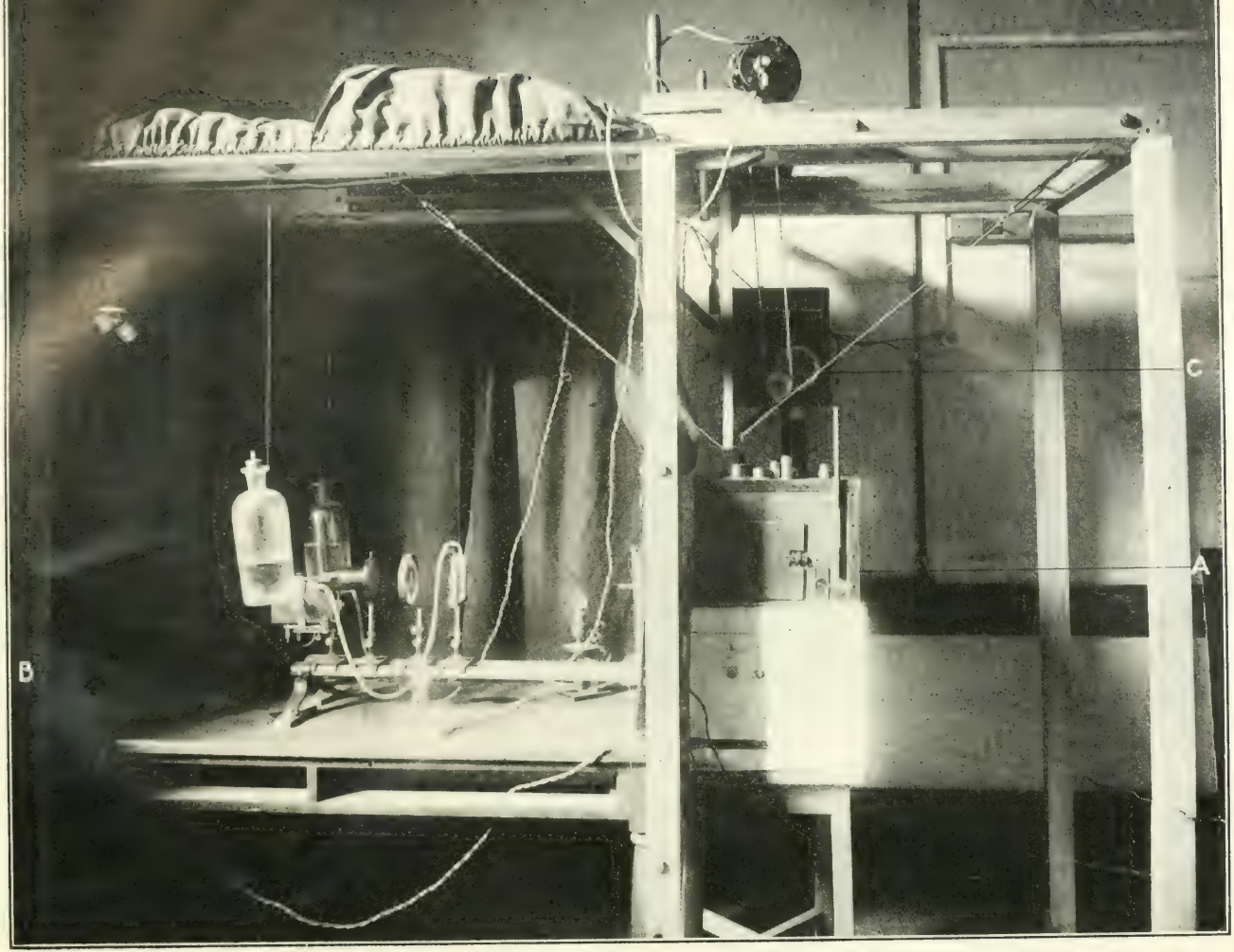
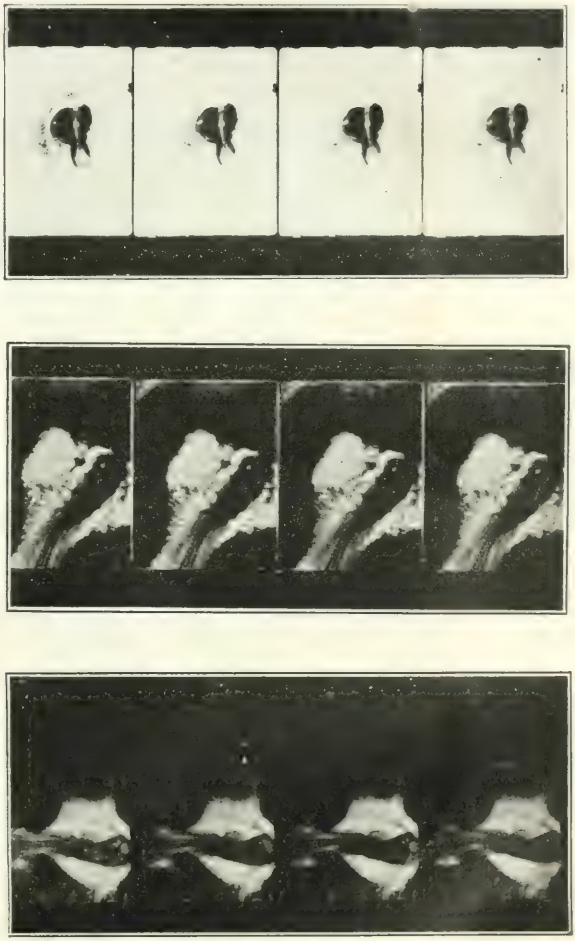


Fig. 3. Apparatus: (*a*) The modified incubator; (*b*) optical bench; (*c*) cinematograph.

In adapting the cinematograph to photomicrography the necessary changes are but two; first, a replacement of the lens by a metal tube; second, the substitution of a pulley and interlocking gear-wheels for the ordinary hand-cranking



Figs. 4, 5 and 6.—Early stages in the development of the chick blastoderm; magnification from 2 to 5 diameters.

device. The gear-wheels, two in number, are of different sizes. The larger is screwed fast to the shafting that operates the mechanism within the camera, while the smaller, to which is riveted a small pulley, engages the larger wheel at one

side. This reducing gear is so arranged that one revolution of the pulley gives one revolution of the rotary shutter within the camera, the equivalent of one picture.

The camera is held in place over the microscope by firmly clamping it to a slotted plate. This plate is suspended over the incubator and held in place by means of a heavy skeleton framework, to which it is attached above, and reinforced below with guy wires. The framework extends the whole

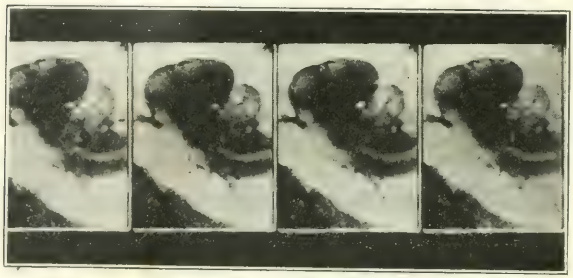


Fig. 7.—Heart in so-called loop stage; magnification from 5 to 10 diameters.

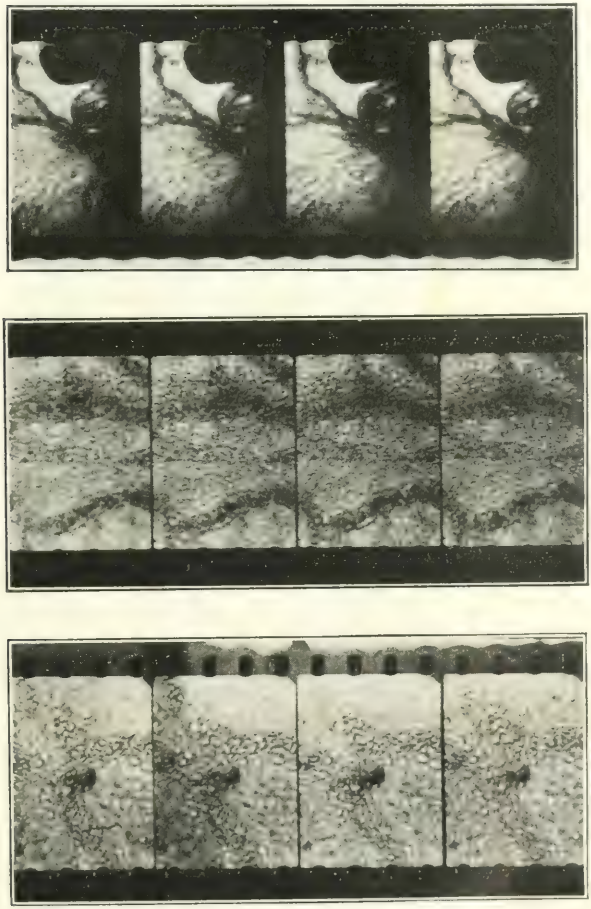
Fig. 8.—Heart in so-called irregular loop stage, magnification from 5 to 10 diameters.

length of the optical bench and incubator, and is, wherever possible, bolted to that part of the apparatus, thereby acquiring a very high degree of rigidity.

The cinematograph is operated by means of a small, slowly running motor, situated immediately above the camera and connected by belting. As the motor is governed by a rheostat, the number of pictures taken per second and the length of exposure as well are capable of being varied within very wide limits.

METHOD OF USE

The method of photographing with this apparatus is the following: After the desired field in the specimen is found, the cinematograph is clamped to the slotted



Figs. 9, 10 and 11. Primitive circulation in area pellucida; magnification from 10 to 100 diameters.

plate. The camera is then so centered that the eyepiece of the microscope is in a direct line with the center of the exposed film. All extraneous light is excluded by means of the tube, which, as already mentioned, is

attached to the lens-board of the camera and when in position surrounds the draw-tube of the microscope. The rays from the arc-light having been carefully centered and cooled, the image to be photographed is brought to a sharp focus on the film. Having adjusted the diaphragm beneath the microscope to give the proper illumination of the field, and having determined the number of pictures per second necessary to record all

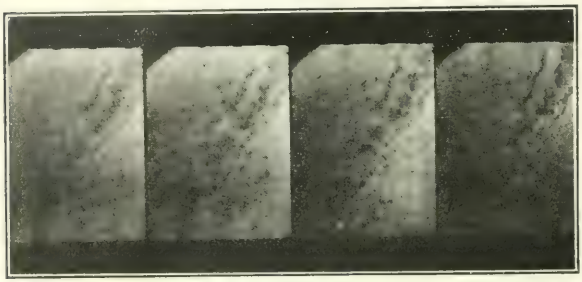


Fig. 12.—Primitive circulation more highly magnified; magnification 200 diameters.

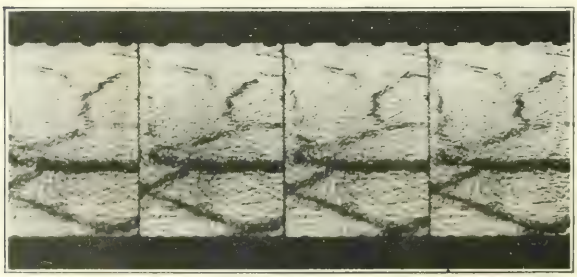


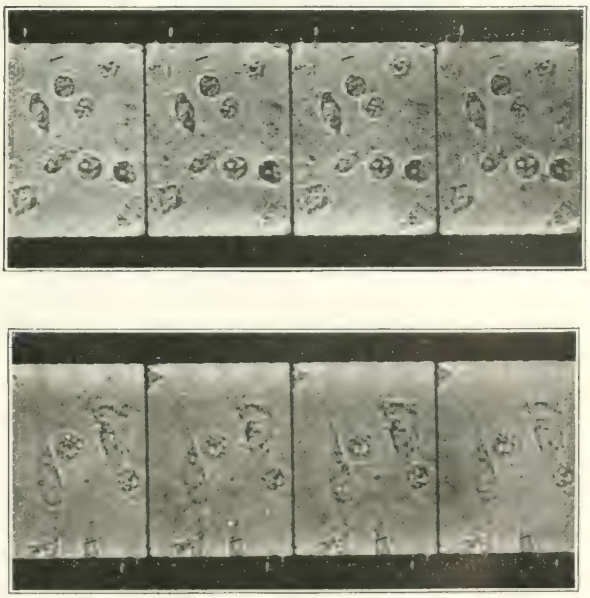
Fig. 13.—Circulation in frog's bladder, magnification 100 diameters.

the movements of the particular specimen under observation, the rheostat is next adjusted to the proper speed and the motor started.

The speed at which our pictures were taken has varied with the specimen; those of the beating embryonic heart, for example, were taken at the rate of about eight per second, while the movements of the cells may be clearly shown when taken at a rate not exceeding one or two per second. The movement of blood-cells in the circulation, however, is exceedingly rapid, and a speed

of twenty-five per second is only sufficient to take those cells in the more slowly-moving blood-currents.

Fig. 14.—Outgrowth of connective-tissue cells in a tissue culture: magnification about 25 diameters.



Figs. 15 and 16.—Ameboid movement of mesenchymal cells: magnification 500 diameters.

In order to show the possibility of this work the accompanying photographs have been reproduced from a few of our films.

The pictures are divided into three groups:

1. The chick embryo in various stages of its development.

2. The primitive circulation in the area pellucida as well as the circulation in the bladder of a frog.

3. The movements seen in the embryonal connective tissue-cell as well as those of the ameba.

The first three embryos in Series 1 (Figs. 4, 5 and 6) show the action of the embryonic heart in the very

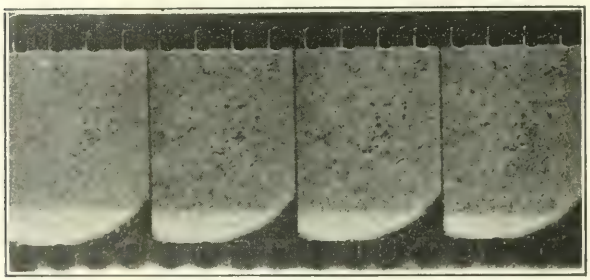


Fig. 17.– Streaming granules in a recently divided connective-tissue cell; magnification about 500 diameters.

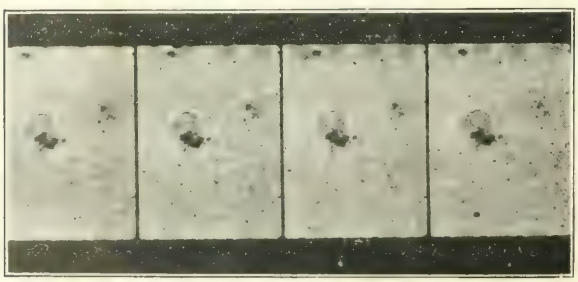


Fig. 18. Ameba, magnification about 500 diameters.

early stages of its development, before the onset of the primitive circulation. These embryos vary in age from twenty-six to thirty-six hours' incubation, and have a magnification of from two to five diameters.

In the fourth picture (Fig. 7), of an embryo of from forty to forty-four hours, there is to be seen the heart in the so-called loop stage, with a beginning primitive circulation.

The next picture (Fig. 8), at from forty-four to forty-eight hours, shows the heart in the irregular S-shaped

stage. The heart at this stage of its development may be seen forcing the blood through its lumen, not as a continuous but as an interrupted stream, the interruptions being synchronous with the contractions of the heart.

The first of Series 2 (Fig. 9) shows a well-marked primitive circulation in the area pellucida as well as the passage of blood through the heart, followed by two moderately low-power pictures (Figs. 10 and 11) of a primitive circulation as found in the area pellucida of a chick embryo of from forty-eight to fifty hours.

The third (Fig. 12) is a somewhat higher-power picture of the primitive circulation taken from the same general region, showing the blood-stream with the individual blood-cells more clearly defined.

The final pictures (Fig. 13) of this group are of the circulation as seen in the bladder of a frog, and show at the same time the peristalic wave of the unstriped muscle.

In Series 3 is shown, first (Fig. 14) the outgrowth of connective-tissue cells in a culture made from the heart of an embryo chick. This is followed by mesenchymal cells, the activity of which is shown (Figs. 15 and 16) by the output of pseudopods, clearly seen in the first two sets of pictures. In the fourth group (Fig. 17), in addition to the pseudopod formation, there is a marked streaming of granules within the protoplasm. The final picture (Fig. 18) is that of an ameba, and is included in this group on account of the similarity of its movements to those of the mesenchymal cell.

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THE POSSIBILITIES OF PRESERVING THE INTEGRITY OF POTENTIAL BODY CAVITIES BY THE USE OF A FOREIGN BODY TO PREVENT ADHESIONS[1]

By FREDERICK PRIME, Jr., M. D., New York City
From the Laboratory of Surgical Pathology, Department of Surgery, College of Physicians and Surgeons, Columbia University

THE possibility of trying to preserve the integrity of the potential body cavities has been a fertile field for investigation ever since our modern laboratories have made possible observations along such lines.

So many evil consequences are the direct result of such cavities as the abdominal, the cerebral, and the joints being partially or completely obliterated, that anything which will alleviate such a condition will be of the greatest benefit to many unfortunate sufferers.

Much has been done in the study of this sequel to many of our commonest operations, and various methods have been introduced in an endeavor to avoid any trauma at the time of operation which might result in adhesions.

In the majority of cases these cavities are obstructed by new connective tissue, forming adhesions, which is the direct result of some inflammatory condition, or a trauma such as may follow an injury or an operation in this region and many efforts have been made to find some substance which, when introduced, will prevent the apposition of surfaces by clots and hinder connective-tissue growth between them.

Adhesions, as we know them, are formed by the growth of new connective tissue, which has been stimulated into increased activity by some irritation, often such as must necessarily take place at the time of operation, no matter how skillfully it may be done, for it is well known that the less handling and injury done at operation, the greater are the chances that no adhesions will be formed. Any injury done to the delicate tissues at the time of operation must result in some reaction, and an exudate be thrown out, or it may be that there is a slight hæmorrhage, so small as to pass unnoticed or demand no attention. The wound being closed, the sides of the cavity may thus easily be fixed one to the other by the exudate or the blood, which

[1] Read before the New York Academy of Medicine, February 6, 1913.

Fig. 1.

forms a framework through which connective tissue grows rapidly, and more or less obliterates the cavity.

By various experiments it has been found that connective tissue does not grow unless it has some network or support along which to

Fig. 2.

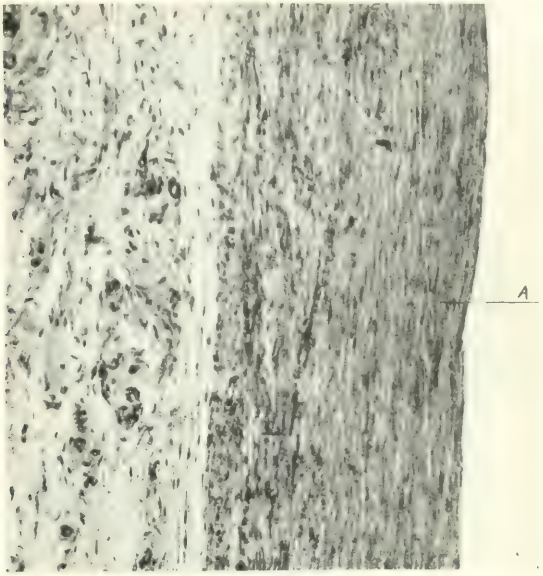

Fig. 3.

climb, and that it is unable to grow into dead spaces or elsewhere without some means of support.

The solution, naturally, to such a problem, after due care has been taken to minimize as far as possible the injury done at the time of operation, is the finding of some material which will prevent the obliteration of the cavity by the growth of connective tissue, when introduced against opposing surfaces, or in such places where blood clot or fibrin can act as a network along which these cells may grow.

The present investigation was undertaken in order to continue

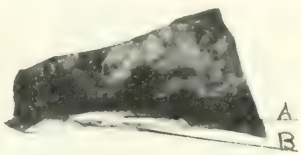

Fig. 4.

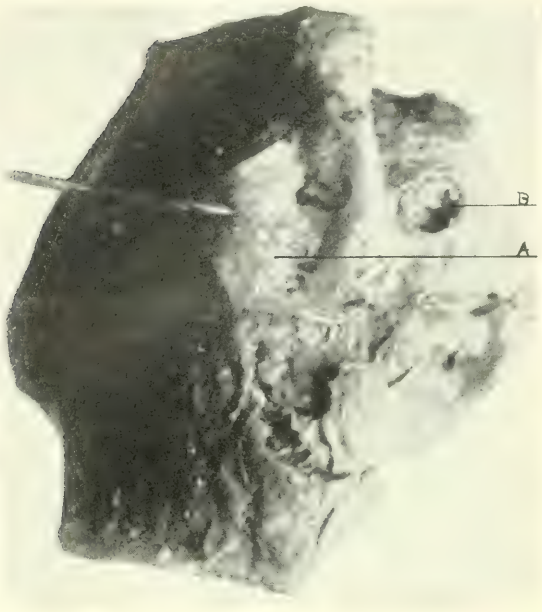

Fig. 5.

a series of experiments conducted some years ago in regard to adhesions, especially as found within the cranial cavity, in which it was shown that the best results were obtained where no foreign substance

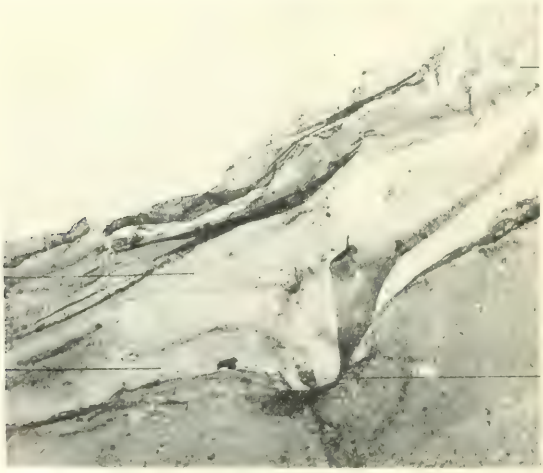

Fig. 6.

Fig. 7.

was used and a stream of warm saline solution allowed to flow gently over the operative area.

This, however, was far from giving an ideal result, and it was felt that in order to obtain a more favorable one, it was necessary to find, if possible, some substance which, when introduced and allowed to remain indefinitely, would be a truly non-irritating body. The foreign materials which have previously been used have all been

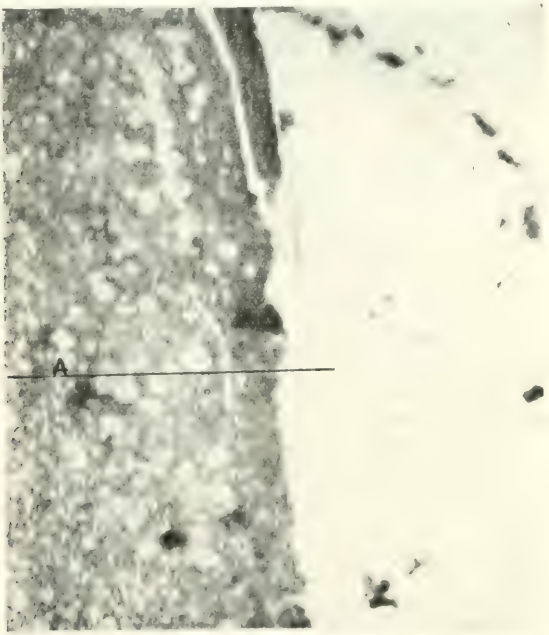

Fig. 8.

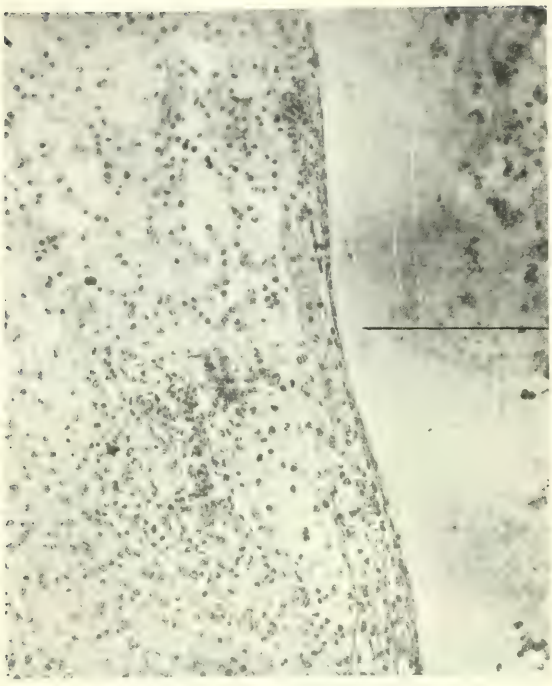

Fig. 9.

irritants to a greater or less degree, and usually caused a worse condition than the one they were supposed to cure, stimulating rather than retarding the growth of connective tissue. Especially has this been the case where such substances as rubber tissue, gold and silver foil, or animal tissue as egg or cargile membrane, were used. Absorbable materials seem less desirable practically, though theoretically they should be more so, than non-absorbable ones, for upon disintegration the remaining particles act as a supporting framework for the production of new connective-tissue growth.

The ideal material should therefore be a non-absorbable, non-irritating foreign body, and it has been with this in mind that the following experiments have been undertaken. The finding of such a material, which would answer the purpose and at the same time be easily handled and readily sterilized, and yet of a sufficient degree of thinness, was a difficult one. From previous experiments, and in going over the ground already covered, celluloid seemed in many ways to be the ideal type of substance, the drawbacks to its use being the inability to procure it in sufficiently thin sheets, and because the chemicals employed in its manufacture are in themselves irritating.

Fig. 10.

This naturally suggested celloidin, which had been used by Maximow about 1901, and even earlier by Alferow and Sirzow, in experiments on growing connective tissue, and they found it pliable against the tissues and easily made into any desirable form. Though celloidin has been recognized and used for so many years, in a careful review of the literature no record has been found of its having been used in an effort to prevent the formation of adhesions.

To use celloidin it was necessary to obtain it in such a form as to be available for this work, and as nothing of the kind was found on the market it took some time to acquire a method which would produce the necessary sheets.

In making the sheets and tubes used throughout these experiments, a thin etherous solution of German celloidin (Schering) was employed, and after many failures it was found that glass moulds covered with a thin layer of beeswax, and then evenly coated with one or more layers of celloidin according to the thickness required, was the best method of obtaining them.

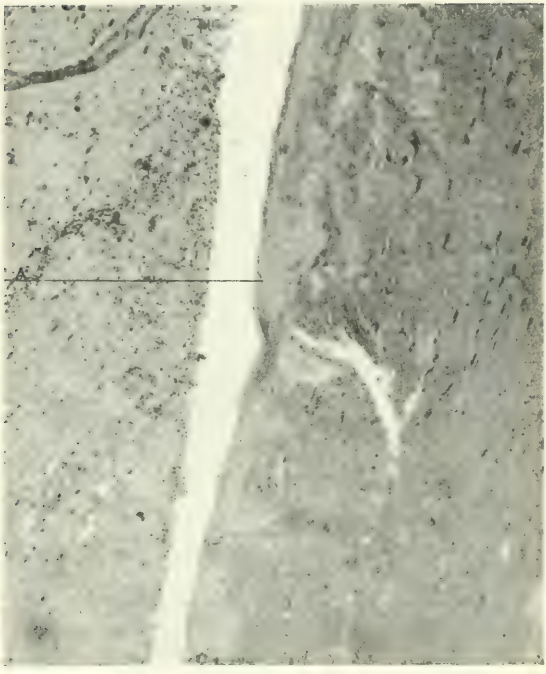

Fig. 11.

The forms being coated, it is essential that the celloidin should be allowed to become absolutely dry and this process must be a slow one at about average room temperature, and several weeks may be required before they are ready to be removed and used. As to their removal, in the case of the tubes it is only necessary to immerse the mould for a second in boiling water, when the wax will quickly melt, allowing the celloidin to be slipped off, after which they should be placed in chloroform for some hours in order to remove any wax which may have remained adherent. The same method has been used in removing the sheets, but it has been found more satisfactory, when they have been thoroughly dried, to peel them carefully off the mould and then remove the excess of wax with chloroform, after which they are ready for sterilizing before using. By this method there is practically no limit to the size of the sheets and tubes which can be made, and the former can be made of tissue-paper-like thinness, though at the same time they are extremely tough and pliable (Fig. 1).

As thus prepared, the tissue can be readily sterilized either by boiling in plain water or by wrapping the sheets in paper and sterilizing in the autoclave under pressure. When this latter method is

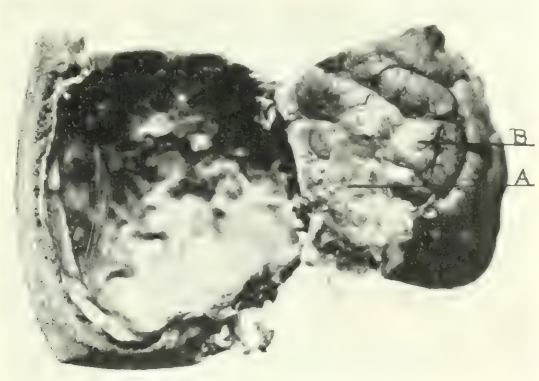

Fig. 12.

used, the greatest care must be exercised to keep the celloidin away from any metal or it will burn, and crumble upon being opened.

One decided advantage in using celloidin sheets is that there is no difficulty experienced in manipulating them when the hands are moist, as is the case with cargile membrane, and they can be moved if once placed on a moist surface, where other materials will only stick and tear, and can be wrapped with ease around nerves and tendons, or placed within the abdominal or other body cavities, if necessary, without any difficulty; but they cannot be stitched.

In order to ascertain whether celloidin could be used to advantage in situations where adhesions are most likely to form, the brain, joints and nerves were selected as the most suitable places to introduce the

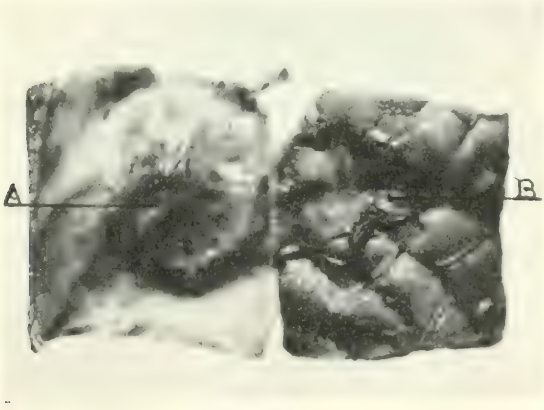

Fig. 13.

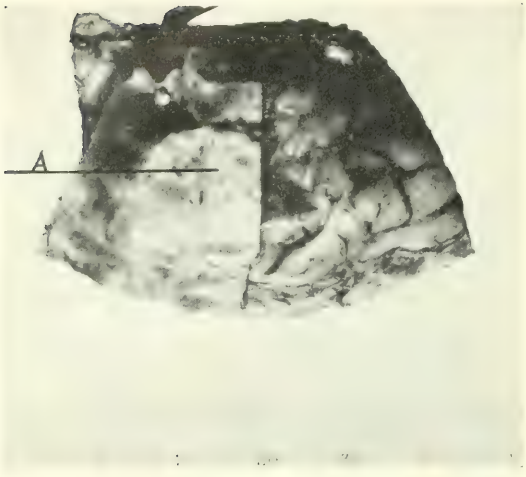

Fig. 14.

tissue, with the results which I shall presently bring before your notice.

In a number of cases small celloidin tubes were implanted in the cortex to see whether connective tissue would grow into them or be attached to the side, in other cases the sheets were used in places where it was known that without them adhesions would be found

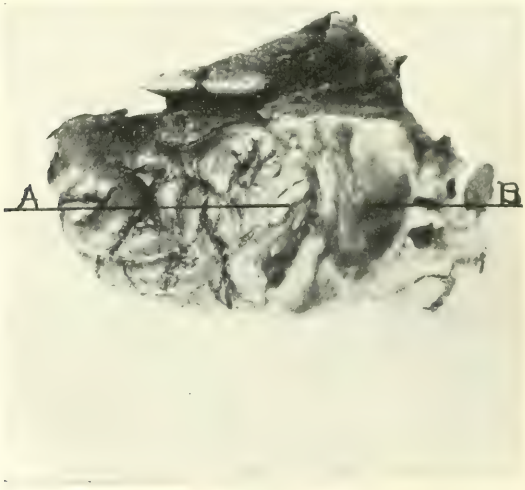

Fig. 15.

Fig. 10.

soon after operation. In the experiments rabbits and dogs were used, and, as irritation was more profusely responded to in the brain than elswhere, more experiments were undertaken within the cranial cavity than in other situations.

The technique was as follows: In the cranial cases, the majority had an osteoplastic flap reflected, though in a few the bone was removed and any profuse hæmorrhage from the skull checked by a small quantity of Moorhof-Mosetig wax. The dura was then opened, and in some cases the surface of the cortex sponged with gauze to favor the formation of adhesions. Following this, a small piece of celloidin sheet was introduced between the dura and the brain, and the former, in the majority of cases, was closed with fine silk sutures.

When tubes were implanted, on exposing the cortex, the least vascular area was selected, a blunt probe introduced into the brain substance, and along this tract the tube inserted. The brains exposed in some of the rabbits were so small that it was often difficult

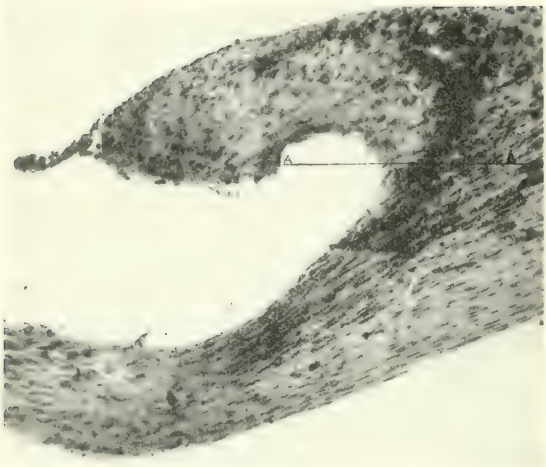

Fig. 17.

to select what was supposed to be a silent area, so that in one or two cases signs of hemiplegia ensued, which did not, however, interfere in the least with the experiment.

Where the operation was upon a tendon or nerve, they were cut and sutured, and then surrounded by a tube or tissue.

A foreign body, placed anywhere within the tissue of the body when it is non-absorbable, we expect to find quickly covered with a dense mass of adhesions. With celloidin, however, the picture is somewhat different, as may be seen in Fig. 2, lent me by Dr. William C. Clarke, where a large celloidin capsule was placed deeply under the skin of a dog and allowed to stay for two months. At the end of that time it was found covered with a dense sheath of connective tissue, which was smooth and glistening, but without the slighest trace of adhesions of any sort to the capsule. The microscopic section of this connective tissue showed that it was dense and made up of structural cells and fibers, and was denser against the capsule (Fig. 3). This same condition was found in practically all the cases where celloidin was used. Within the cranial cavity there would be no adhesions if a piece of celloidin were placed beneath the dura, but between the celloidin and the dura, and often between the celloidin and the brain, a connective-tissue membrane would form which was smooth and glistening and free from fibers binding it to the underlying tissue. The same thing was found along the nerves, and even when, as a mass, the celloidin was found wrapped in the omentum, the same glistening membrane was found, with the celloidin quite free within it.

The explanation of these phenomena seems to be that at the time

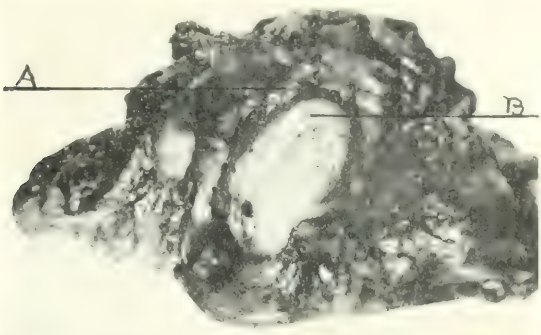

Fig. 18.

of operation, when, for example, the dura is opened and manipulated, there is sufficient injury done to this delicate membrane to set up a reaction, and cause an exudate to be thrown out. The celloidin present prevents this from reaching the underlying surface, so that when the connective tissue begins to grow into this exudate, it simply grows along the surface of the celloidin and the dura, thickening the latter. The same thing may occur along the under surface of the sheet, if any trauma has been done to the cortical surface, but at the same time the celloidin has prevented any adhesions forming between the dura and the brain (Fig. 4).

In the first series of the following experiments, rabbits were used, and celloidin sheets placed under the dura, and the animals allowed to go from two to six weeks before being killed. At the end of two weeks, autopsy showed no adhesions between the celloidin and the dura, and though there was a slight infection and exudate under the celloidin, the latter was free and not bound down (Fig. 5).

Where four weeks had elapsed between operation and autopsy there were dense adhesions found between the dura and the overlying muscles, the bone having been removed at operation, but between the celloidin and the dura there were none, though the latter was somewhat thickened, nor were there any between the celloidin and the cortex, which was perfectly normal in appearance.

At five weeks there was no gross examination made, but the specimen was decalcified and embedded, and cut in serial sections, and there were no adhesions present, as may be seen by the photomicrograph (Fig. 6).

The results at the end of six weeks were the same, and the gross specimen showed a cortex which was free from any adhesions to the overlying sheet (Fig. 7).

Fig. 19.

In three rabbits celloidin tubes were implanted in the cortex, and in the first one, examined at the end of five weeks, the microscopic section showed no adhesions to the tube, and though in some cases there were evidences of hæmorrhage from the injury done by inserting the tube, it did not show evidence that the tube had acted in any way as an irritant (Fig. 8). It must be borne in mind that the reaction due to any injury such as inserting the tube will cause the appearance of some round cells which are not due to irritation or infection from the tube.

The second tube was left in place over eight weeks, and upon microscopic examination, with the exception of a small localized abscess, the surrounding tissue was normal with no reaction such as is commonly seen around a foreign body (Fig. 9).

In the case where the third tube was inserted, there were signs of a hemiplegia soon after operation, and the rabbit died three days later with symptoms of intracranial pressure. At autopsy there was no visible hæmorrhage under the dura, as was suspected. The specimen was cut in serial sections with the tube in position, and some hæmorrhage was found along its tract, and within it were some fibrin and blood-cells, but no reaction to the tube itself (Figs. 10 and 11).

Five experiments of a similar nature were done on dogs and in most of the cases the specimens removed at autopsy were preserved in gross, with the following results:

Where the celloidin was placed under the dura, at the expiration of two weeks it was found in one case that the dura was thickened and adherent to the cortex except just over the celloidin sheet, where there were no adhesions at all (Fig. 12). In the second case, killed after the same interval, the celloidin had in some manner worked its

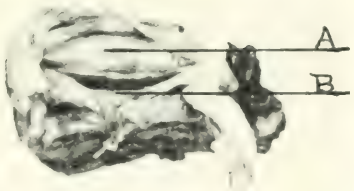

Fig. 20.

way on top of the dura, which had not been sewn. The dura in this case was adherent to the cortex, but there were no adhesions between it and the overlying celloidin, or the latter and the brain at the point where the dura was open.

At the end of four weeks, in another case, the dura was found greatly thickened over the sheet, but was free and not bound down in any way to the cortex.

After five weeks the condition was the same found in the other cases, a thickened dura and no adhesions, and between the dura and cortex a glistening layer of connective tissue, quite unattached (Fig. 13).

One dog was kept for five months, at the end of which time the celloidin was found encapsulated on both sides with a layer of connective tissue, which was smooth and glistening, and formed a pocket in which the celloidin lay, and yet left the cortex quite free from adhesions (Figs. 14 and 5).

Two animals were used as controls. One was kept for two months, celloidin being placed over, not under, the dura. In this the dura was thickened under the sheet, and densely adherent to the cortex, but not to the overlying muscles. The second was kept alive for five months and the adhesions between the cortex and the dura, where no celloidin had been interposed, were so dense that the cortex was torn in attempting to remove the dura (Fig. 15).

Tubes were inserted into only two dogs. The first died at the end of seven days, of distemper. Serial sections were made of the cortex with the tube in position, and it was found free from adhesions to the brain substance, with some fibrin a short way into the tube. The brain walls showed no irritation (Fig. 16).

In the second case the tube was left in position for three months. At the end of this time no irritation could be seen.

In all of these cases where the dura was thickened, microscopic section showed connective-tissue cells laid down (Fig. 17), which is quite different from the thin normal dura as it is ordinarily seen.

An effort was made, without any success, to obtain adhesions within the shoulder-joint of dogs. In a number of cases the shoulder-joint was opened and the articular surface of the head of the humerus scraped gently with a curette, and celloidin placed between the two articulating surfaces. In the only case where there was satisfactory healing the celloidin had remained in position, and the joint cavity was entirely free from adhesions at the end of ten days (Fig. 18).

In cases of nerve suturing, the results were most satisfactory. A nerve in the foreleg which had been cut across and sutured with fine silk, and a celloidin tube slipped over the point of suture, at the end of ten days showed no evidence of the nerve being bound down, but was free in the tube (Fig. 19), and at the end of six weeks another which had been wrapped with a piece of celloidin tissue showed a sheath of connective tissue which had formed over the celloidin, leaving the nerve healed and free beneath it (Fig. 20).

From the foregoing results the following conclusions have been deducted:

1. It is possible to use a non-irritating, non-absorbable foreign body in the potential body cavities and not produce adhesions.

2. Celloidin in properly prepared forms is such a substance.

3. In places such as the joints and cranial cavity, as well as around nerves, it can be used to prevent the formation of new adhesions or hinder the re-formation of old ones.

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THE FUNCTION OF THE PERIOSTEUM IN BONE TRANSPLANTS, BASED ON FOUR HUMAN TRANSPLANTATIONS WITHOUT PERIOSTEUM, AND SOME ANIMAL EXPERIMENTS[1]

By CLARENCE A. McWILLIAMS, M.D., New York City

THOROUGHLY to appreciate some of the questions involved in the subject of bone transplantation, it will be necessary to consider briefly the three different views of recognized authorities as to the manner of regeneration of a graft.

1. The universally accepted view in Germany is that of Axhausen, who maintains that the bone in a graft always dies, is absorbed and is re-formed from the periosteum, which alone remains living in transplants.

2. The second view is that of Macewen of Glasgow, who says that the bone in a graft is reproduced from the proliferation of osteoblasts, derived from the osteoblasts within the bone of the graft itself, and its regeneration takes place independently of the periosteum, whose only function is that of a limiting membrane, which prevents the spread of the osteoblasts into the surrounding tissues.

3. The third view is that of Murphy, who says that the graft is not osteogenetic but simply osteoconductive. Provided that it be in contact at one or both extremities with other living bone, the graft acts simply as a scaffolding for the growth of the capillaries with their osteogenetic cells as they advance from the living, contacting extremities into the graft. The periosteum takes no part in the actual reproduction of new bone. To analyze Dr. Murphy's transplantations a little further, I may say that I have gone through all of his reported transplantations with the object of finding out just what was done with the periosteum in each procedure. In a number of cases this was not stated. In all such instances I have ascertained from him in writing exactly what was done with the periosteum. In not a single instance did he omit to transplant grafts with more or less of their covering periosteum. From a practical point of view, I should judge that he does not know just what would have happened had he transplanted grafts without their periosteum, so that his conclusions as to the function of the periosteum are only conjectural.

The same may be said of Albee's work along this line. He agrees

[1] Read in the Section on Surgery, American Medical Association, Minneapolis, June 1913. From the Surgical Research Laboratory of Columbia University.

with Macewen and Murphy that the periosteum is unimportant so far as the life of the graft is concerned, yet, notwithstanding this view, he has never done a human transplantation without the periosteum, although he has performed 160 transplantations in all. It would strike the unprejudiced critic that the theories and practice of these men do not quite agree. Macewen and Albee have argued that, because they did not consistently obtain bone as a result of transplanting periosteum alone, consequently the periosteum has little function when it is left attached to grafts. Early in my work along this line

Fig. 1. Case 1. Rib grafted into a defect in lower jaw. A, rib.

it struck me that the proper angle from which to view this question of the function of periosteum could be obtained only from transplanting grafts of various sizes with and without their periosteum, since this is exactly what we do in our human transplantations. We do not transplant periosteum alone. The former is precisely what I have done and I think that I have definitely and conclusively proved, as you will later see, that the periosteum has a very important function in maintaining the nutrition of the graft.

When I looked over the results in a general way of both my animal and human transplantations, at first sight many of them seemed contradictory. On further analysis, however, it appeared as though all of these contradictions could be readily explained if we looked at it in this light: The life of a living graft, whether transplanted in the human being or in animals, whether transplanted with or without its

periosteum, is probably entirely dependent on its being supplied with sufficient blood to keep it alive. I am inclined to believe that this will be the solution of the many vexed, theoretical considerations of bone grafting, and will explain the apparent contradictions and varying results obtained in bone transplantations. To particularize further, practically every bone graft made with its covering periosteum will live and grow if asepsis be attained. The question is, What will happen to grafts without their periosteum? The answer that I have obtained from my work along this line is this: The life of a graft without periosteum will depend entirely on its blood-supply. If it obtains sufficient blood, then it will live and grow; if not, then it will die. A small graft without periosteum will have a better chance of getting sufficient blood

Fig. 2. Case 2. Rib grafted into inguinal hernia without periosteum.

to its cells than a large one without its periosteum; but in either case there is always some doubt as to whether the graft will live, if it be without its periosteum. The periosteum on a graft does one of two things, or possibly both, namely:

1. By its presence it so favorably influences the nutrition of the graft, that is, increases the blood-supply to its cells, as to keep it alive, or

2. In case the bone cells in the graft die from insufficient nourishment, the periosteum supplies living cells to the graft, by means of which the bone is regenerated.

I can see no possible reason why the living cells in a graft should die if they be provided with sufficient blood. I hold this to be the function of the periosteum when attached to a graft. I hope to justify the soundness of my position in the illustrations.

HUMAN TRANSPLANTATIONS

Case 1. In November, 1910, I removed half the lower jaw from a lad of 12 for a large giant-cell sarcoma. Fifteen months later I grafted into the defect in the lower jaw a piece of rib which was entirely stripped of its periosteum. One end of the rib was beveled and this was sutured to the freshened edge of the re-

maining half of the jaw. The wound healed by primary union. Fig. 1 shows the rib in position. Five months later, a röntgenogram showed an entire disappearance of the grafted rib. There never was any discharge from the scar.

The result in this case is a contradiction of Dr. Murphy's second conclusion, that "bone, with or without the periosteum, transplanted in the same individual and contacted at one or both ends with other living, osteogenetic bone, always becomes united to the living fragment, and acts as a scaffolding for the reproduction of new bone."

Had I split the rib longitudinally so as to afford a better blood-supply to the bone-cells of the graft, it seems to me that it might have lived, or had I transplanted the rib with periosteum covering half its surface.

Case 2. The patient was a man, aged 32, into whose inguinal canal I trans-

Fig. 3 K, Case 3.

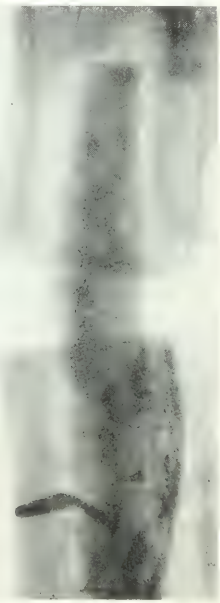

Fig. 3 P, Case 3.

planted a piece of a rib to strengthen a hernial repair. Fig. 2 shows the rib in position. A röntgenogram, four months later, showed that the rib has entirely disappeared.

This case is corroborative of Dr. Murphy's conclusion that bone transplanted into muscle always dies and is ultimately absorbed. That this statement is not always true, however, will be seen later from some animal experiments. Had I split this rib likewise, I believe that the consequent better blood-supply to its cells would have kept it alive.

Case 3. The patient was a man, aged 32, who had received a compound fracture of the left humerus, a month before he came under my care. He was

operated on immediately after the accident in another institution and the fracture was wired. This was followed by violent sepsis. Two sequestra were picked out of the wound the next day after the man came under my care. The patient had a musculospiral paralysis, indicated by the wrist-drop. The result of the application of a Lane plate to the fracture, after sawing off the ends of the fragments, was still non-union.

Five months after the previous plating, on account of non-union, I grafted into the medullary cavities of the fragments, which were first reamed out, a section from a subperiosteally resected rib. After the rib was in position, there was some side-to-side rocking of the fragments on each other, due to the fact that the rib was evidently a trifle too short. On this account I applied a Lane plate. It was then thought best to unite the divided musculospiral nerve. The ends were dissected out and it was found that there was a defect of an inch and a half to be bridged over. This was accomplished by cutting out of the distal extremity a segment, which was turned up to meet the proximal end, to whose freshened

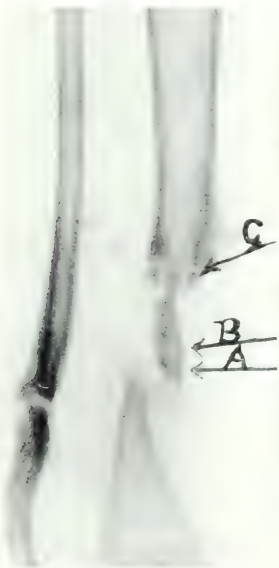

Fig. 4 B, Case 4.

Fig. 4 E, Case 4.

A, section for microscopic examination taken from tibia; B, section for microscopic examination taken from graft; C, graft.

edge it was sutured with chromic gut. The result of this bone grafting was very disappointing. A picture (Fig. 3 K) after nine months showed no proliferation whatsoever of its bone and there was no better union than at the time of the grafting.

That the musculospiral nerve was regenerating was shown by the fact that sensation had returned in the distribution of the nerve, although there was no motor power as yet. Nine months after the rib grafting, I removed (Fig. 3 K) the remains of the grafted rib. A section of it under the microscope showed that it was undergoing rarefying osteitis and that it consisted mostly of dense fibrous tissue with scattered areas of bone. The medullary cavities of the fragments were reamed out (Fig. 3 P), and into them was inserted a graft, taken from the

front of the tibia with periosteum on two of its sides. A nail was inserted in its upper end to prevent its riding up into the medullary cavity.

The result of this grafting was unfortunate. There was infection of the wound, requiring the removal of the nail and the dead graft. So that his condition was not improved.

Case 4. This is an exceedingly interesting case of non-union in a birth fracture of both bones of the leg in a child, aged 17 months. The non-union I attributed to insufficient immobilization of the leg. I cut down on the fracture and, after freshening the pointed extremities of the fragments, there resulted a defect of an inch and a half in the tibia, which made transplantation of bone necessary. A graft from the opposite tibia was chiseled out with its periosteum

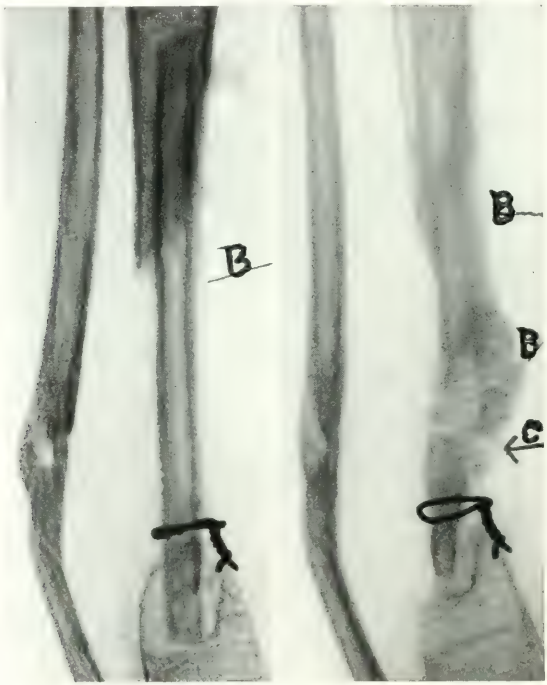

Fig. 4 I, Case 4. B, new bone in a thin plate along periosteum. Taken two months after operation, shows beginning new bone being formed.

Fig. 4 K, Case 4. B, new bone from periosteum; C, fracture.

attached. The periosteum was then deliberately peeled off the graft with the object of confirming Macewen's view of its unimportance. One end of the graft was pointed and it was wedged into the medullary cavity of the upper fragment, and the lower side of the transplant was spliced to the side of the lower fragment with chromic gut. The subsequent union of the wound was by first intention. Fig. 4 B shows the condition six months after the grafting. It was very disappointing to see the tibial graft gradually melt away in the tissues, so that, after six months, it was not more than one quarter its original size. Since union was by first intention, there seems no way of accounting for the death of the graft save on the basis of a lack of periosteum, or of a deficient blood-supply, or both.

Fig. 4 E shows the condition six months after the previous grafting operation, at which time I transplanted 5 inches of the opposite, healthy fibula into the medullary cavities of the fragments, as seen. It was transplanted with the periosteum covering its entire circumference, and this was not split in the slightest. The result shows that this splitting of the periosteum on the graft is entirely unnecessary and accomplishes nothing. Below, a wire-encircling suture was used to hold the graft in place. On the right one sees the defect made in the

Fig. 4 M, Case 4. Fig. 4 P, Case 4.

fibula. Fig. 4 I shows new bone being formed along the periosteum of the fibula graft, as a thin plate, two months after grafting.

Fig. 4 K was taken six months after the transplantation. To my mind it seems to imply the necessity of revising Dr. Murphy's conclusion that a graft is not osteogenetic in itself. A fracture has taken place in the mid-third of the transplant, due to the fact that the child kicked off the splint one night. We see that this fracture of the graft itself is consolidating nicely under an enormous callus. If we accept Dr. Murphy's conclusion, we must believe that all this callus came through the graft from the contacting old bone of the neighboring stumps.

I do not accept this view for a moment, but believe that the callus arose either

from the periosteum or from the bone of the graft itself. Along the outer surface of the graft we see at least a quarter of an inch of new bone evenly distributed. Were this new bone formed from the contacting ends of the old bone of the stumps, it seems to me that it would shade off, becoming less the farther we went from the old bone. As a matter of fact, the greatest amount of new bone

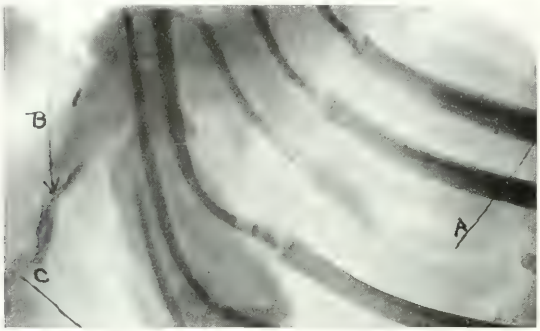

Fig. 6. Experiment 2. A, resected rib; B, periosteum with new formation of bone after six months, three weeks transplanted into the abdomen; C, transverse section for microscopical examination.

is at the fracture spot in the graft, which is at some distance from either contacting extremity of old bone. Fig. 4 K shows that the transplant (June 9, seven months after the grafting) has continued to develop. The upper part of the graft has become as large as the opposite healthy tibia. The fragments below the fracture, due to the inhibitory influence of the wire, have not well developed. In addition, the wire is eroding the bone of the graft. I removed this

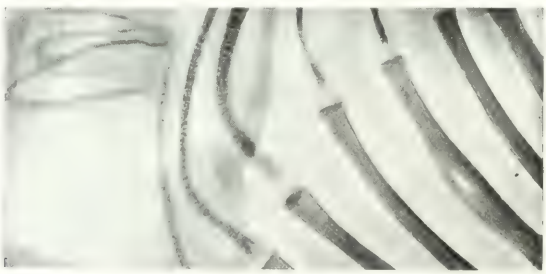

Fig. 7 A, Experiment 3. Middle of left rectus, sheath divided and strips laid on muscle, separated from each other, sheath sutured over these. Picture shows rib entirely reformed and growth together of bones in abdomen.

wire June 14, 1913, with the object of preventing the cutting through of the graft.

Fig. 4 M shows that, six months after the transplantation, there is no filling in of the defect in the fibula with new bone since all the periosteum was removed with the graft. To have new bone fill up a defect it is absolutely necessary to leave either some bone or else some periosteum in place bridging the defect.

Fig. 4 P shows the result two months after the removal of the wire. The

lower fragment which was being eroded by the wire has markedly developed and the fracture is consolidating nicely.

At the recent surgical congress in New York, Dr. Murphy said, "In bone transplantations there is but one absolute essential, other than rigid asepsis, and that is that the bone graft must be closely connected at some one point at least with living, freshened, osteogenetic tissue." From the preceding human transplantations, I judge that there is one other essential, and that is the presence of the periosteum.

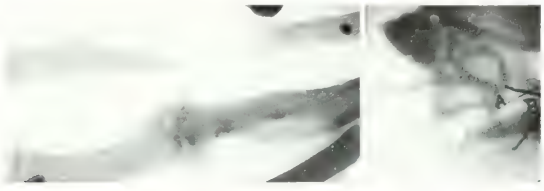

Fig. 8 A. Fig. 8 B.

Fig. 8 A, Experiment 4. Two ribs subperiosteally resected. Shows perfect reformation of one rib and imperfect reformation of other.

Fig. 8 B. A, section for microscopical examination; B, new bone.

ANIMAL EXPERIMENTS

Experiment 2. Fig. 6 shows the condition six months after the resection of the whole diameter of a rib together with its periosteum. The defect has not at all closed in. The periosteum was stripped bluntly from the bone section and this strip of periosteum was transplanted into the abdomen.

Result: A transverse section was cut six months later through this periosteum, which was felt to be bone. A photomicrograph of this section gives a picture of normal, healthy, living bone surrounded by a closely investing con-

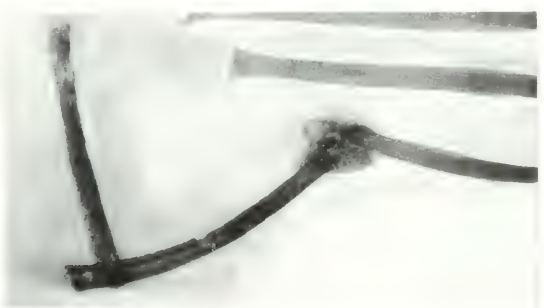

Fig. 9 A, Experiment 5. The two transplants are shown, the upright or second transverse rib shows beginning absorption and also the absence of union between it and the adjacent transplant. The first transplanted rib shows an excessive callus and solid union due to periosteum.

nective-tissue capsule. There are no osteoclasts present nor any round cells suggestive of either a destructive or an inflammatory process. I shall not go into a description of these microscopic sections but shall give you simply the results of them. This result shows that transplanted periosteum may produce living bone.

Experiment 3. This animal was killed eight months after the subperiosteal

resection of a section of a rib (Fig. 7 A). This rib without periosteum was then split into longitudinal strips and these were transplanted into the abdomen.

Result: The defect in the rib from which the periosteum was not removed has become entirely filled in with new bone (Fig. 7 A). The strips transplanted into the abdomen have remained intact, have grown together, and have increased in size. A photomicrograph of a section of this abdominal transplanted bone shows bone perfectly alive, not undergoing absorption, and containing well-preserved and normal marrow. I attribute the living of these transplants to the fact that the splitting of the original rib allowed sufficient blood to get to the bone-cells.

This and the following experiment are refutations of Dr. Murphy's conclusion, in which he says that bone, with or without its periosteum,

Fig. 10. Experiment 6. A, four resected ribs all filled in. Removed without periosteum. B, transplanted rib all that is left of two grafts. Section from this spot for microscopic examination.

when transplanted into the soft parts and not in contact with living bone, always becomes absorbed.

Experiment 4. This dog was killed six months after subperiosteal resection of two ribs. The ribs without periosteum were broken up with the rongeur into the smallest possible fragments and these were transplanted into the abdomen.

Result: The defects made in the two ribs have acted differently one from the other (Fig. 8 A). The upper defect has about half filled in with new bone while the lower has formed a complete new rib, almost double its normal size. The fragments (Fig. 8 B) into the abdomen have lived, have increased in size, and have amalgamated into one homogeneous piece. A photomicrograph of a section, taken from these abdominal grafts, shows that the graft is perfectly alive, and contains normal marrow and normal blood vessels. There are no osteoclasts present nor any signs of a destructive or degenerative process.

The last two experiments clearly demonstrate that the life of a living graft depends entirely on its receiving sufficient blood to keep it alive, and nothing else. This was made possible by the splitting of the grafts

which allowed sufficient blood to get to their cells. The next two pictures more clearly bring this point out, both being the same experiment.

Experiment 5. In this animal, killed after one month, two ribs were subperiosteally resected. One of the ribs was transplanted into the abdomen, into

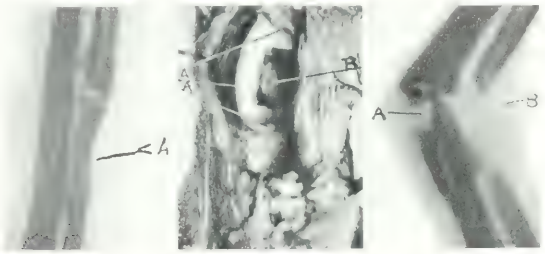

Fig. 13. Fig. 16. Fig. 17.

Fig. 13, Experiment 9. A, pieces of graft.
Fig. 16, Experiment 12. A, edge of periosteum; B, new bone.
Fig. 17, Experiment 13. A, fracture; B, defect in bone not filled in because of removal of periosteum with the bone.

a defect made in the last rib, after the periosteum of the last rib was divided and separated but not removed. The second rib, also without its periosteum, was sutured to the end of the first rib, and both ribs in contact were buried in the abdomen.

Result: The first rib (Fig. 9 A) has healed in perfectly to the defect made in

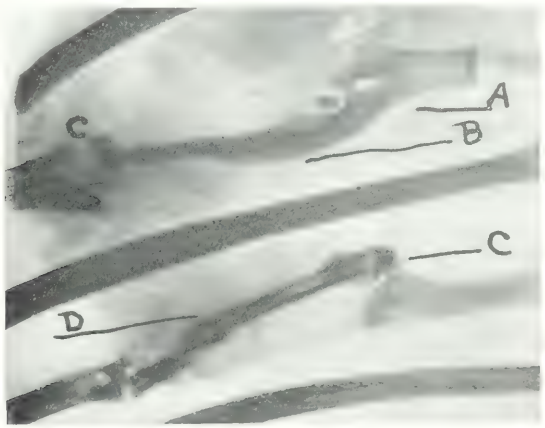

Fig. 18, Experiment 14. A, solid union here; B, section taken for microscopic examination; C, no union due to respiratory motions of chest, notice periosteal outgrowths; D, section taken for microscopic examination. Union here.

the last rib by a massive callus formed at the point of junction, evidently due to periosteal bony growth. There is no union or new bone formed whatsoever between the two transplanted ribs, owing, as I believe, to an insufficient blood supply to their cells and to the absence of periosteum to re-form the bone. In

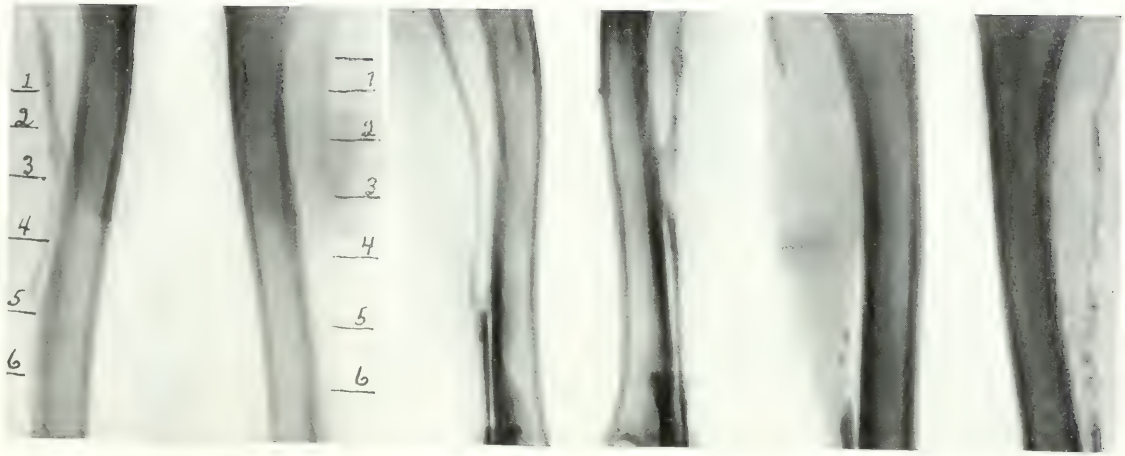

Fig. 19. Experiment 15. Points of section. Fig. 20. Experiment 16. Fig. 21. Experiment 17.

addition, the second rib shows beginning absorption, evidenced by its honeycombed appearance. The picture indicates that an entire graft on an entire graft, neither having any periosteum, will not live.

Experiment 6. This is the same experiment as the preceding. The animal was killed six months after four ribs were resected without their periosteum. One of the ribs without periosteum was sutured to a defect in the last rib, a second rib was sutured to the end of the first, and both ribs were buried in the abdomen. Fragments from another rib, which was divided into as small pieces as possible, were strewn along both the transplanted ribs.

Result: Fig. 10 shows that all the four defects created in the ribs have become completely filled in with new bone, owing undoubtedly to the presence of the periosteum, which had not been removed. The picture further shows that the second attached rib, as well as about half the first rib, have become entirely absorbed. Some of the fragments have become attached to the sides of the remaining part of the first rib.

The last four experiments are a convincing demonstration, it seems to me, of the fact that it is simply and solely a question of a sufficient blood-supply which determines whether a graft will live or die, and not a question of whether the graft be in contact with living bone or not, as Dr. Murphy maintains.

Experiment 9. This animal was killed five months after one inch of the radius, together with its periosteum, was removed. The periosteum was then scraped off the fragment, which was split into as small pieces as possible with the rongeur. These small fragments were replaced, filling in the defect in the radius.

Result: The small fragments (Fig. 13) have all remained alive and have coalesced into one piece, which has become united to the ends of the radius fragments.

This experiment illustrates the influence of a good blood-supply on grafts.

Experiment 12. This dog was killed twenty-four days after the periosteum was excised from a wide area of the humerus. Within this area was chiseled away a section of bone, extending into the medullary cavity, and the marrow and endosteum were thoroughly curetted away.

Result: Fig. 16 shows that the cavity made in the bone is irregularly filled in by new bone, the medullary cavity being quite obliterated by this newly formed bone. The periosteum can be seen ending at quite a distance from this cavity, and by no stretch of the imagination can one think that it had anything to do with the formation of new bone. Likewise with the endosteum. The new bone must then come from the bone-cortex itself, resulting from the efficient blood-supply from the nutrient artery. From these cases and experiments, we see that to have new bone fill in a defect it is necessary to have either some periosteum covering it or else at least a thin layer of bone bridging over the defect.

Experiment 13. The last statement made is exemplified in this animal, which was killed six months after the removal of an inch and a half from the radius, together with its periosteum. The animal was killed because he fractured the ulna, immediately opposite the defect in the radius.

Result: Fig. 17 shows that there has not been the slightest attempt to fill in the defect in the radius with new bone, owing, as I believe, to there being no periosteum left to produce it. If Macewen and Murphy are right in their views, why was this defect not filled in with bone coming from the ends of the fragments?

Experiment 14. This animal was killed three months after the removal of

two inches of bone from two ribs, each with its corresponding periosteum. Each single fragment was transplanted into the defect made by the removal of the other fragment, each being completely covered by its periosteum. They were both held in place, end to end, by sutures passed through drill-holes in their extremities and through drill-holes in the ends of the fixed ribs.

Result: One end (Fig. 18) of each graft has not united to its corresponding extremity of the rib, owing to the respiratory motions. The other ends are solidly united to the fixed rib. In addition there is irregular production of new bone at various points on the grafts, due to periosteal growths. This result shows the great influence the periosteum exerts in preserving the life of grafts. Microscopic sections of each of these transplants show that they are both actively alive.

Experiment 15. This animal died six weeks after 2½ inches were resected from each fibula with the periosteum attached. Into the right fibula defect was transplanted the left fibula graft without any of its periosteum, the transplant being first divided into six fragments. Into the left fibula defect was transplanted the right fibula graft, which was previously divided into six fragments, on each of which was left its corresponding periosteum.

Result: The fragments without periosteum (Fig. 19) into the right fibula defect have become almost entirely absorbed. There is just a faint line indicating the little that remains of the grafts. In startling contrast to this is the result in the left leg, into which defect the fragments were transplanted with the periosteum on each fragment. These individual grafts have not been absorbed in the slightest; on the contrary, they have grown together and have increased in size. It is not possible to escape the conclusion that this good result in the left leg is entirely and absolutely due to the presence of the periosteum. If the result depended simply on the contact with the neighboring, living bone of the stumps, as Murphy maintains, why did not the fragments without periosteum in the right leg live?

Experiment 16. Fig. 20 shows the condition eight months after sections were taken from each fibula, from each of which the periosteum was carefully scraped. Into the left fibula defect was transplanted the entire, undivided, right fibula fragment without periosteum in contact with the ends of the living stumps. Into the right fibula defect was transplanted the left fibula fragment without periosteum, which was first divided into two longitudinal strips, these being placed side by side in contact with the ends of the living stumps.

Result: The two fragments without periosteum (Fig. 20), comprising the graft into the right fibula defect, have lived perfectly and have united together and to the ends of the old stumps. In addition there is proliferation of new bone, uniting the graft to the neighboring tibia. In contrast to this is the result in the left defect. This graft without periosteum, transplanted entire, has very poorly united to the ends of the old stumps, and the transplant appears, from its poor shadow and irregular honeycombed appearance, to be absorbing. I attribute this difference in the result to the fact that the splitting of the graft allowed a much better blood-supply to reach the bone-cells of this graft than was possible in the case of the graft which was not split.

Experiment 17. Fig. 21 is very instructive and illuminating, and warrants close study. It was taken eight months after sections of bone were taken with the periosteum from each fibula. Into the left fibula defect was transplanted the right fibula graft, which was first broken up into the smallest possible pieces, the periosteum being thoroughly scraped from the small fragments. The pieces completely filled in the defect and were in contact with the ends of the stumps of the old bone. Into the right fibula defect were transplanted fragments of the left fibula graft with periosteum still attached to each small graft.

Result: Fig. 21 indicates the great value of having periosteum on grafts. The small fragments without periosteum in the left defect have become largely

absorbed, being much reduced in number. Those which remain are isolated, are not united, and appear to be absorbing. In marked contrast to these are the fragments transplanted into the right defect with periosteum. They have become united to each other in most instances, have increased in size, and produce the deep shadows of healthy, living bone.

This experiment is another instance of the fact that contact with living bone does not seem to be the important element which Dr. Murphy has attributed to it. This experiment is a further proof of the statement, emphasized by me, that without periosteum there is always uncertainty as to whether grafts, even though they be small, will acquire enough blood to keep them alive. With the periosteum on the grafts one is sure of their living, I believe, as the result of one or two things happening: either a sufficient blood-supply, or a resupply to the grafts of living cells, derived from the periosteum, which reproduce the bone.

Experiment 18. A transplantation in which, into a defect made in the left fibula, were transplanted fragments obtained from the right fibula, each covered with its periosteum. Into the right fibula defect were transplanted fragments from the left fibula section, from which all the periosteum was first carefully scraped away.

Result: The result in this dog confirmed the view that the life of grafts depends simply and solely on the blood-supply which they obtain. The fragments with periosteum into the left fibula defect appear to have lived perfectly, to have increased in size, and to have solidly united with each other and to the ends of the old, living stumps. The same process occurred on the right side, into which defect fragments without periosteum were transplanted.

Experiment 19. In this experiment, into a defect made in the left fibula were grafted fragments, taken from a section of the right fibula, from which all the periosteum was first carefully scraped away. Into the defect made in the right fibula were transplanted fragments with periosteum on each piece, taken from a section of the left fibula.

Result: The fragments transplanted without periosteum into the left fibular defect have almost completely disappeared. The fragments transplanted into the right defect with periosteum have remained living, have markedly increased in size, and have largely united to each other and to the ends of the old stumps. The feature to be particularly noticed in this picture is the pronounced increase in size of the right-sided grafts, denoting the active life of the transplants and also the fact that, although the middle section of the graft on the right side does not seem firmly united to the neighboring transplants, yet this middle section appears to be as actively alive as the sections which are in intimate contact with the ends of the old stumps; all of which goes to show that contact with living, old bone has not the influence on the subsequent life of grafts that Dr. Murphy has attributed to it. Far more, as I have repeatedly tried to emphasize, do I believe that it is solely a question of sufficient blood getting into the graft.

The accompanying two tables give the results obtained in all of these transplantations.

From Table I it may be seen that 93 per cent of all transplantations made with the periosteum were successful, while but 48 per cent of those made without the periosteum were successful.

TABLE I.—RESULTS OF GRAFTS WITH AND WITHOUT PERIOSTEUM

			Totals	
With —	Animal	Human	No.	Per Cent
Successful	13	1	14	93.3
Unsuccessful	1	0	1	6.7
Without —				
Successful	12	0	12	48
Unsuccessful	9	4	13	52

TABLE II.—GRAFTS WITHOUT PERIOSTEUM: RESULTS OBTAINED IN TRANSPLANTATIONS WITHOUT THE PERIOSTEUM ACCORDING TO THE SIZE OF THE GRAFTS.

			Totals	
Small fragments—	Animal	Human	No.	Per Cent
Successful	7	0	7	50
Unsuccessful	7	0	7	50
Larger, single grafts—				
Successful	5	0	5	41
Unsuccessful	3	4	7	59

From Table I it is seen that 48 per cent of all the transplantations made without periosteum were successful. From this I reason that there must be some other factor present, making for the success of these grafts, than the mere covering of the periosteum, and this I infer must be the presence of a sufficient blood-supply to keep them alive. Had the periosteum been on the grafts, it may be safely said that they would practically all have survived. Just exactly what the influence is which the periosteum exerts, it seems difficult to state positively. Whether its presence affords a better blood-supply to the grafts, or whether the periosteum, in the event that the bone-cells in grafts have died, supplies fresh cells to the grafts and from these regeneration takes place, must for the present remain unsettled. That the blood-supply is more than likely the important point is seen from Table II. It goes without saying that a small fragment will get a sufficient blood-supply to its cells more readily than a single, large piece, hence is more likely to remain living. This important fact is proved in Table II, in which it is shown that 50 per cent of the transplantations in which small fragments without periosteum were grafted were successful, while but 41 per cent of the transplantations in which single, larger pieces without periosteum were grafted were successful.

I think, in conclusion, that I have demonstrated that both Macewen and Murphy are mistaken in their conception of the lack of function of the periosteum in maintaining the life of grafts. It seems to me likewise that Murphy is misled in his conception that the graft is not osteogenetic in itself. This fact is clearly seen in Experiments 3, 4, 17 and 19. He says that the graft is simply osteoconductive of cells into the graft from the contacting extremities of the neighboring old bone. If this be the case, why should so many of the grafts without periosteum have died in which this contact was thoroughly carried out?

CONCLUSIONS

The conclusions from the preceding human transplantations and animal experiments are:

1. If the periosteum be excised from an area of bone and a section of bone within this area, going into the medullary cavity, be removed

(not, however, involving the whole diameter of the bone) and the endosteum scraped away as well, then it is found that the cavity fills in perfectly with new bone, which of necessity must come from the old bone alone. This is probably due to the fact that the nutrient artery is intact.

Proofs: Human transplantation, Case 4, in which the defect in the tibia filled in perfectly; Animal Experiment 12.

2. If a section of the whole diameter of a bone be removed, then the bone will regenerate between the ends of the fragments if the whole or a part of the periosteum be preserved bridging the defect.

Proofs: Animal Experiments 3, 4 and 6; Baskirzew's human case (see my Conclusion 6). The subsequent reformation of the ribs after human empyema operations performed subperiosteally.

3. If a section of the whole diameter of a bone be removed, very little subsequent filling in of this defect by bone will take place if the entire periosteum has been removed from between the ends of the fragments.

Proofs: Animal Experiments 1, 2, 13, 15 (right), 17 (left), 19 (left) and 20 (left). Human transplantation, Case 4, in which a great part of the fibula with all its periosteum was removed. There is very little of this shaft which has been reformed. In case that we wish to have a bone reform, it seems necessary to leave some of its periosteum in position. This is brought out by Frangenheim[1] and von Hacker.[2] They each took pieces from the fibula, comprising the whole thickness of the bone together with all the periosteum covering these fragments. There was no subsequent reformation of bone in the defects. Frangenheim, consequently, recommends that in taking a section from the fibula, a small strip of periosteum, from which the fibula will be reformed, be left on the interosseous membrane.

4. Provided a graft be living and taken from the same patient, its future life depends on an efficient blood-supply, irrespective of the periosteum or whether it is in contact with living bone or not. This conclusion I wish to emphasize strongly.

Proofs: Animal Experiments 3, 4, 8, 16 and 18.

5. If minute fragments of a living graft be transplanted, then the periosteum may be disregarded because the osteoblasts in the fragments may not die because of an easy access of blood into them, and the pieces may grow and coalesce and not become absorbed. My statistics show that 50 per cent of such transplantations will be successful.

Proofs: Animal Experiments 7, 9, 16 and 18, also Macewen's human case. Thus, in his book he narrates the case of a boy, the whole of the diaphysis of whose humerus he was compelled to remove for necrosis. There was no subsequent osseous deposition. Fifteen months later, two wedges of bone were excised from another patient 6 years of age, affected with anterior curves. These were cut into minute fragments, quite irrespective of the periosteum, and were then

[1] Frangenheim: Arch. f. klin. Chir., 1907, [illegible]

[2] Von Hacker: Quoted by Streissler, Beitr. z. klin. Chir., 1910, lxxi, Part 1.

deposited in the muscular sulcus in the boy's arm. Two months later, a portion of new bone, an inch in length and three-quarters of an inch in thickness, was found firmly attached to the upper fragment of the humerus. Here all the grafts proliferated, grew to one another and also to the extremity of the proximal portion. Two other wedges of bone of larger size than the first and broken up into fragments were similarly dealt with and inserted two months subsequently to the first graft, and a third couple were placed in position five months after the first. These all fused together and to the condyles of the humerus and filled the gap in the arm to the extent of 4 inches. It is now thirty years since the shaft of the humerus was rebuilt, and, during that period, the man has depended on his physical exertions for his livelihood. He is now an engineer's pattern-maker. Other cases in which minute fragments of bone have been successfully transplanted are on record. Thus, Vallas[1] reported the case of a woman on whom he had operated five months previously for a pseudo-arthrosis of the tibia. At the operation, he found that the ends of the fragments touched at only a small point, due to the slender form of the inferior fragment. He then removed from the rounded extremity of the upper segment ten or twelve bony chips which he placed in the defect between the bones. The periosteum and soft parts were then sutured about these chips. Primary union resulted and in six weeks consolidation was complete and the patient left the hospital. Barth[2] reports two successful cases in each of which he transplanted two fragments, taken from the tibiæ, each covered by periosteum. In both of these cases, the fragments grew and proliferated and filled in the defects perfectly. Even though one is safer in transplanting small fragments without the periosteum than larger ones (compare Experiments Nos. 16 and 18), at the same time the small fragments may die if they are without the periosteum (compare Experiments Nos. 15, 17, 19 and 20).

6. If a larger piece of living bone be transplanted, then it is safer to leave the periosteum attached to the graft to assure its future reformation, since otherwise the osteoblasts in the bone of the graft may die because cut off from their nutrient artery. The periosteum survives because of its sufficient blood-supply from the surrounding tissues; its inner surface forms osteoblasts which themselves proceed to reform the bone of the graft, in the event that the osteoblasts within this have died from a deficient blood-supply. My statistics show that 41 per cent of transplantations are successful in which large grafts without periosteum are grafted.

Proofs: Previous Cases (human) 1, 2, 3 and 4; Animal Experiment 14. For the sake of being explicit, I wish to state that this conclusion does not imply that the periosteum is unconditionally necessary for the growth of transplanted bone. It merely asserts that one is much surer of the grafts living if the periosteum is present on the graft. There are comparatively few cases on record of grafts having been made without the periosteum. I presume that this is due to the fact that it is not customary to report non-successes. I have come across the following two cases which were successful after transplanting grafts without the periosteum: Baskirzew[3] reports a case which illustrates two things: first, how well the defect in a bone made by removing its entire diameter will fill in, provided the periosteum be left; and, second, how well a graft may live and grow at times even when transplanted without its periosteum. Thus, he removed a 9 cm. piece without its attached periosteum, from the diaphysis of a

[1] Vallas: Lyon méd., 1894, lxxvii, 606.

[2] Barth: Arch. f. klin. Chir., 1908, lxxxvi, 860.

[3] Baskirzew: Deutsche Ztschr. f. Chir., 1912, cxiii, 524.

fibula for the purpose of a transplantation. Four years afterward, in the place where the fibula was removed, a stout, although uneven, fibula resulted. The piece from this fibula was transplanted in a defect made in the ulna for the removal of a supposed sarcoma in which not only the bone but also its periosteum was entirely removed. The graft in the ulna without periosteum, transplanted into a bed not lined by periosteum, grew in perfectly and consolidated with the upper fragment, a fact due, as I believe, to sufficient blood having been supplied. The second case is that by Wetherill.[1] On September 28, 1912, he grafted a segment, three and a third inches long, from the opposite tibia into a defect in the tibia resulting from osteomyelitis. The graft was taken without its periosteum. A röntgenogram taken after six months shows that the graft has lived and has increased in size.

7. A graft on a graft, neither having a covering periosteum, will not live, even though one graft be in contact with living bone.

Proofs: Animal Experiments 5 and 6.

8. Periosteum when transplanted alone into the soft parts may produce living bone.

Proof: Animal Experiment 2. The remarkable vitality of transplanted periosteum has been demonstrated by Grohe[2] and Morpurgo,[3] the former showing that it is capable of preservation for one hundred hours and yet able to be implanted and preserve its osteogenetic powers; the latter showing that the periosteum of a corpse, kept at 15° C., can produce new bone when transplanted after 168 hours.

9. The splitting of the periosteum of a graft, even though the transplant is entirely surrounded on all sides by periosteum, seems to be unnecessary and accomplishes nothing.

Proof: Previous Case 4 (human), in which the fibula graft was transplanted with periosteum on all its sides and this was unsplit. The graft grew beautifully.

10. Altogether sixteen transplantations with periosteum were performed and of these all but one (93 per cent) were successful.

11. Altogether twenty-five transplantations without periosteum were performed. Of these 48 per cent were successful (all in animals), while 52 per cent were unsuccessful. The influence of the blood-supply is demonstrated by the fact that 50 per cent of the transplantations without periosteum, made with minute fragments, were successful, while 41 per cent of those without periosteum, made with large, single fragments, were successful.

12. From a consideration of all the foregoing facts, the conclusion seems irresistible that bone grafts of whatever size, in order that one should be positively assured of their subsequent living, should be transplanted with as much periosteum covering their surfaces as is possible.

I desire to thank Dr. Joseph Blake for the privileges of the laboratory, Dr. William C. Clark for the pathologic examinations, and Mr. Ira Simms, of the Presbyterian Hospital, for the excellent röntgenograms.

[1] Wetherill, H. G.: The Growth, the Death and the Regeneration of Bone. J. Am. M. Ass., 1913, March 2, p. 985.

[2] Grohe: Virchow's Arch. f. path. Anat., etc., Berl., 1899, clv, 428.

[3] Morpurgo: Virchow's Arch. f. path. Anat., etc., Berl., 1899, clvii, 172.

The Periosteum in Bone Transplantations

Is Contact With Living Bone Necessary for the Life of Grafts, and Will Transplanted Periosteum Produce New Bone?

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THE PERIOSTEUM IN BONE TRANSPLANTATIONS

IS CONTACT WITH LIVING BONE NECESSARY FOR THE LIFE OF GRAFTS, AND WILL TRANSPLANTED PERIOSTEUM PRODUCE NEW BONE? *

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Experiments were undertaken to determine, if possible, answers to the above questions. A distinguished authority, Dr. John B. Murphy, has maintained that contact with living bone is absolutely and unconditionally necessary for the subsequent life of grafts. Thus, he expresses himself[1] as follows:

> In bone transplantations, there is but one absolute essential, other than rigid asepsis, and that is that the bone graft must be closely connected at some one point at least with living, freshened, osteogenetic tissue. Also[2] he says: Bone with or without its periosteum, transplanted into the muscle or cellular tissue, practically always dies and is absorbed, when it is free from bony contact.

No proofs, however, were forthcoming to substantiate this conclusion, and it was with the object of helping to decide this question that the following experiments were performed. Figure 1 proves that strips and fragments of bone without periosteum, transplanted into the soft parts, not in contact with living bone, may remain living. This is confirmed by photomicrographs. In Figure 2, one fibula strip without periosteum, which was in contact with living bone (the last rib), is shown to have become absorbed, leaving the three other fibula strips without living bone contact, yet alive and active.

* From the Surgical Research Laboratory of Columbia University, College of Physicians and Surgeons, New York.

1. Murphy, John B.: New York Med. Jour., Nov. 23, 1912, p. 1232.

2. Murphy, John B.: Contribution to the Surgery of Bones, Joints and Tendons, THE JOURNAL A. M. A., April 6, 1912, p. 989.

In Figure 3, the two ribs with periosteum are shown to have lost their contact with the living last rib, yet, notwithstanding, they both remain perfectly alive. In Figure 4, it may be seen that the left fibula *B* with periosteum, transplanted into the soft parts of the right leg

FIG. 1.—Difference between the reformation of the rib when the periosteum is left and when it is removed. (Experiment 1.) Above, defect in rib filled in; below, bone without periosteum persisting in defect with no bony contact.

and not in contact with living bone, remains beautifully alive. Figure 5 shows that the left fibula *B*, with periosteum into the soft parts of the right leg, not in contact with living bone, has proliferated to at least three times its normal size. It is very actively alive. Figures

6, 7 and 8 are roentgenograms of a human transplantation in which a fracture of the graft took place and the way new bone developed about this fracture, to my mind, does away with the idea that contact with other living bone has much to do with the life of grafts.

Conversely, I am prepared to show that even with this contact with living bone, grafts without periosteum may not live. Murphy[3] says:

> Bone with or without periosteum, transplanted in the same individual and contacted with other living osteogenetic bone at one or both of the ends of the transplanted fragment always becomes united to the living fragments and acts as a scaffolding for the reproduction of new bone of the same size and shape as the transplanted fragment, if asepsis is attained.

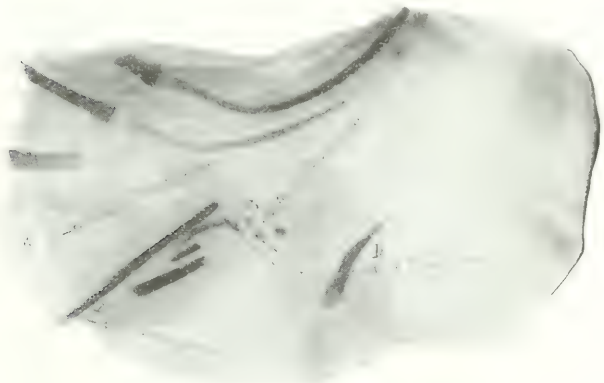

Fig. 2.—Uncertainty of grafts without periosteum (Experiment 35): *A*, strip without periosteum, entirely absorbed; *B*, strip with periosteum alive; *C*, strip without periosteum broken up into six pieces displaced upward; *D*, strip with periosteum alive with production of new bone.

No definite proof has been forthcoming as to the truth of this statement. I have gone all through Dr. Murphy's reported transplantations and find that in not a single instance has he made a transplantation without periosteum being on the transplant. Contact with living bone with Murphy is the keynote of success. To my mind he has overlooked the most important point in bone-grafting, namely, the periosteum. Thus, Figure 9 shows that fragments from the right fibula transplanted

3. Murphy, John B.: Contribution to the Surgery of Bones, Joints and Tendons. Tri. J. A. M. A., April 6, 1912, p. 989.

into the left fibula defect without periosteum, although in contact with the living stumps *A* and *B*, have entirely disappeared, while those fragments into the right fibula defect from the left fibula with periosteum have remained living beautifully. Contact with the living

Fig. 3.—Transplantation of ribs (Experiment 4). Gaps in ribs not filled up, periosteum being removed; A, transplant fractured into two pieces, A¹ being the second and lower piece of A; B, second grafted rib (with periosteum) likewise fractured, B being the small upper part of Bi.

stumps certainly can have had very little to do with the life of the fragments in the center of the right leg transplant. Another roentgenogram showed the same result in an exactly similar operation with the exception that

the fragments in the right leg with periosteum have taken on enormous growth, and who will say that contact with living bone has had any influence on the production of new bone in the central section of the transplanted fragments in the right leg of Figure 9?

Again, in Figure 4 the right fibula without periosteum *A*, transplanted into the left fibula defect, is shown to

Fig. 4.—Difference made by presence or absence of periosteum (Experiment 40): *A*, fibula without periosteum has disappeared, although in contact with old stumps; *B*, fibula with periosteum, though not in contact, has remained living.

have almost entirely disappeared, although this fibula was in contact with the living stumps, while the left fibula with periosteum *B*, grafted into the right fibula defect, yet not in contact with living bone, has remained living. Exactly the same thing is shown to have occurred

in Figure 5, *A* being the position in the left leg of the transplanted right fibula without periosteum. This has entirely disappeared, while *B* is the left fibula with periosteum, not in contact with living bone, transplanted into the right defect. It has grown and enlarged markedly.

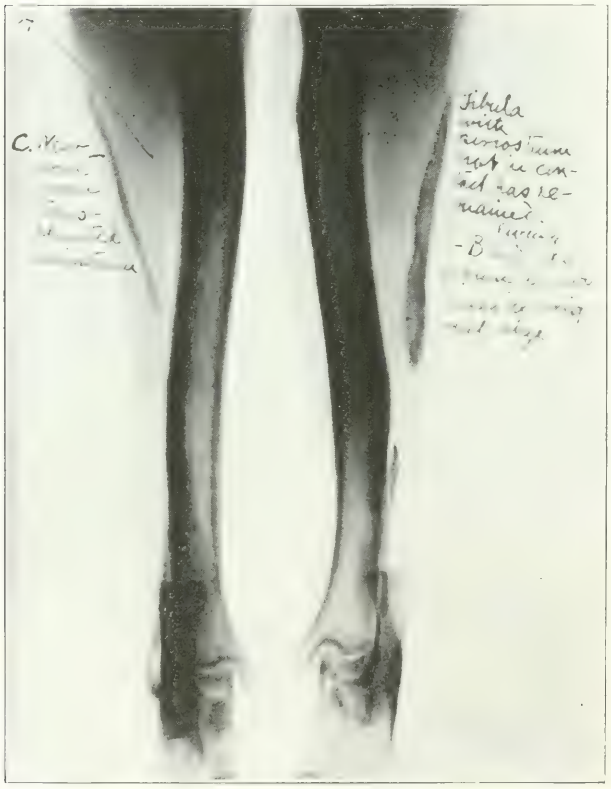

Fig. 5.—Bone transplantations with and without periosteum; *A*, piece of fibula without periosteum has entirely disappeared; *B*, fibula with periosteum not in contact has remained living and has grown to four times its original size; *C*, new bone from transplanted periosteum.

Murphy, Albee and others have had practically 100 per cent. of successes with grafts made with periosteum. My experience is the same, except that I have had one human transplantation with periosteum which became

infected, necessitating the removal of the graft, which should not count in the result, and one animal experiment in which the graft made with periosteum disappeared. I have had 87 per cent. of successes with periosteum on grafts (sixteen cases).

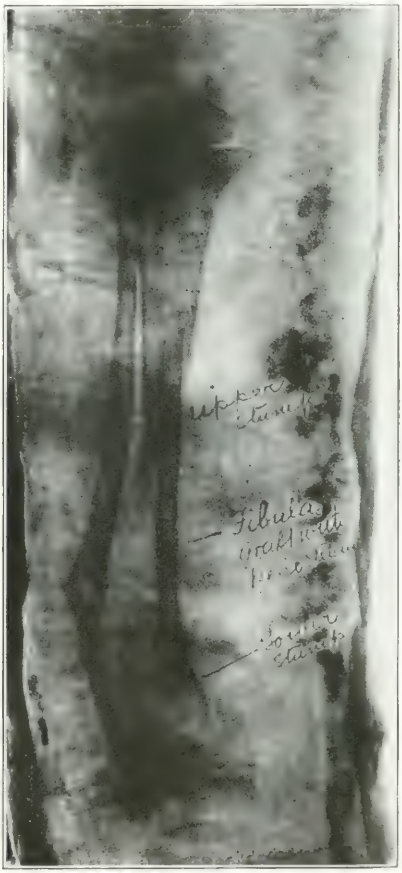

Fig. 6.—Five inches of the opposite, healthy fibula, transplanted into the medullary cavities of the stumps of the old tibia, in a child.

On the other hand, I have had twenty-five transplantations without periosteum, and of these but 48 per cent. succeeded, although there was primary union in all. How can one escape the conclusion that periosteum, next

to asepsis, is the important factor in making for success in bone-grafting? There may be some uncertainty as to just how the periosteum acts, but of its importance there can be no doubt. My conclusion is that it acts by affecting the blood-supply to the grafts and that its osteo-

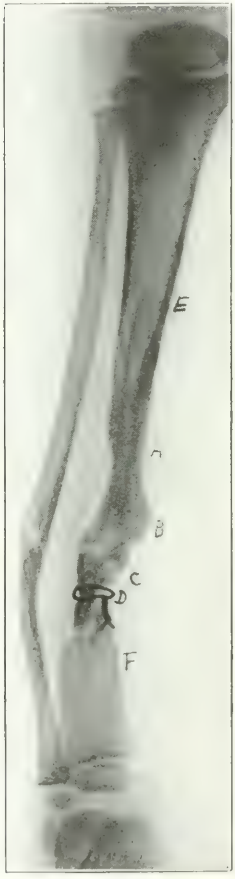

Fig. 7.—Seven months after the grafting operation in the same patient as in Figure 6; *A*, new bone; *B*, callus; *C*, fracture which occurred two months after the operation; *D*, lower fragment of the graft; *E*, *F*, ends of the old bone of the upper and lower stumps.

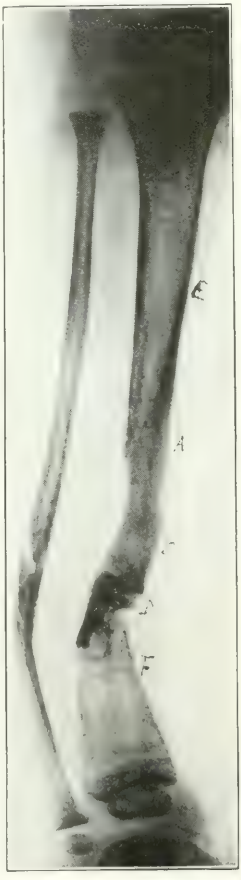

Fig. 8.—Three months after the removal of the wire strand shown in Figures 6 and 7, ten months after the original grafting operation; *A*, new bone; *B*, callus; *D*, lower fragment of graft; *E*, *F*, ends of the old bone of the upper and lower stumps.

genetic function is of secondary importance, else why did 48 per cent. of the grafts without periosteum succeed? The answer, it seems to me, is that the blood-supply of the 48 per cent. of successful grafts without periosteum was sufficient to keep them alive, while in

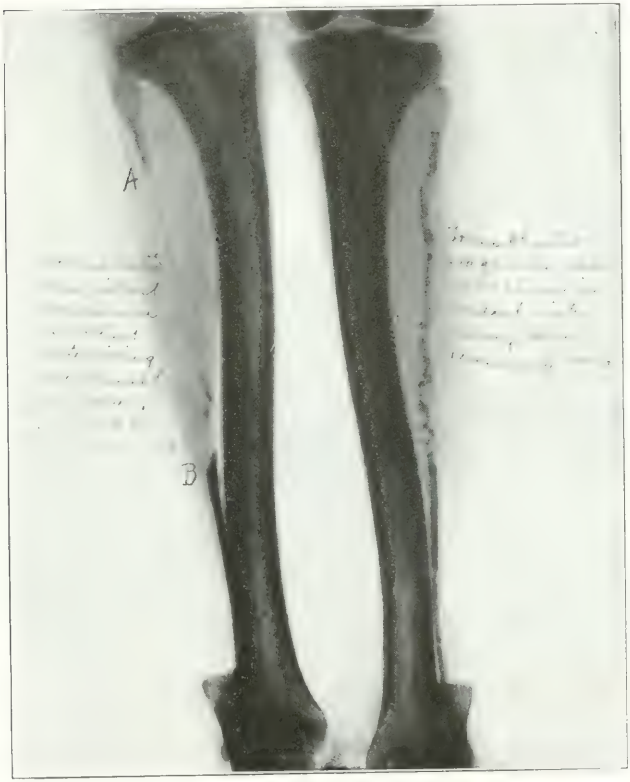

Fig. 9.—Transplantations with and without periosteum (Experiment 7); left fibula defect, *A*, *B*, transplanted bone without periosteum in contact with living bone almost absorbed; the fibula was first fragmented; right fibula defect, transplanted fragments with periosteum in contact with living bone remaining living.

52 per cent. it was insufficient, and hence the grafts died.

REPORTS OF EXPERIMENTS

EXPERIMENT 1 (Dog 172).—This dog was killed eight months after the operation. This experiment, in the first place, indicates the difference between the reformation of the

rib when the periosteum is left and when it is removed. The rib in this dog was subperiosteally removed. It has entirely reformed (Fig. 1). The rib without periosteum was split into longitudinal strips which were transplanted under the rectus sheath of the abdomen. These strips have grown together and they show no evidence of any absorption, not being honeycombed at all. This I attribute to the fact that the splitting of the rib allowed a sufficient blood-supply to get to the bone-cells so that they remained alive. This and the following experiment are a refutation of Dr. Murphy's statement that a bone-graft, with or without periosteum, when transplanted into the soft parts and not in contact with living bone, always becomes absorbed.

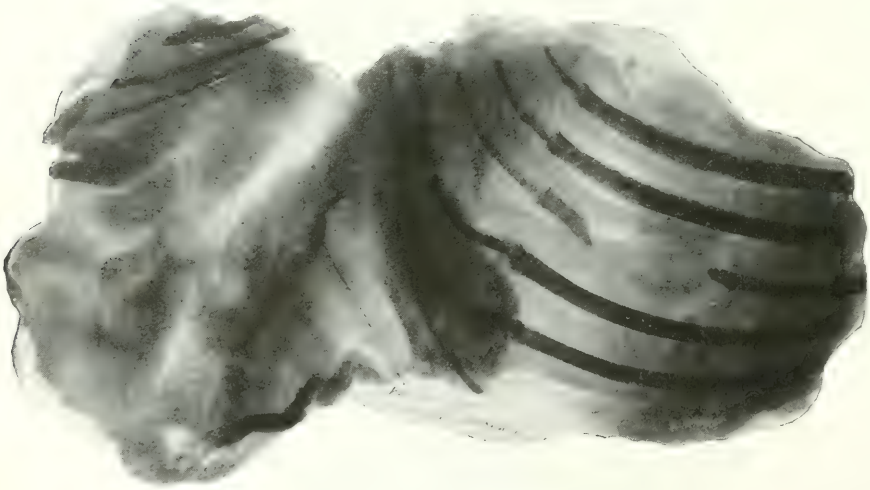

Fig. 10.—New bone from transplanted periosteum (Experiment 9).

A photomicrograph of a section of the abdominal transplant shows that the fragment of bone measures 0.2 cm. in diameter. The bone contains many cells with well-preserved nuclei, and also perfect haversian canals with red blood-cells within them. The bone itself is intact. There are several osteoclasts within lacunae on the margin of the fragment. Many osteoblasts are also present. The marrow is well preserved and contains blood-spaces, many of them filled with red blood-cells. Because of the osteoclasts, there are present destructive processes, but at the same time proliferation is also in progress. The bone seems to be perfectly alive.

Experiment 2 (Dog 19).—The dog was killed six months after subperiosteal resections of two ribs. One rib had entirely

reformed while the other had not quite completely filled in the defect. The ribs without periosteum were broken up into the smallest possible fragments and these were transplanted into the abdomen lying on the muscle. A roentgenogram showed that the fragments had grown together and increased in size. There was no sign of any absorption.

Fig. 11.—Transplantation of fibula sections without periosteum, and transplantation of periosteum (Experiment 10): *A*, right fibula without periosteum has remained living; *B*, left fibula transplanted without periosteum has remained living; *C*, new bone from transplanted periosteum.

A photomicrograph of a section of the bone in the abdomen shows a fragment, measuring 2 by 0.3 cm. This bone fragment is enclosed in a dense connective tissue enveloping capsule. There are many osteoblasts present both on the trabeculae within the bone spaces and also on the periphery of the fragment, especially at either end. There are many well formed

blood-vessels present in the haversian canals. The marrow and adult fat within the bone-spaces also support many blood-vessels, having well-formed walls. The red blood-cells in these blood-spaces are well preserved and distinct. The bone-cells are normal in appearance and their nuclei stain distinctly. There are no signs of degeneration or destructive process present in the sections, which seem perfectly alive.

Experiments 1 and 2 clearly demonstrate that the life of a living graft depends solely and entirely on a sufficient blood-supply. The large grafts were each broken up into smaller pieces, so that blood had easy access to their cells, and hence the grafts lived and increased in size, notwithstanding the fact that there was no periosteum attached to them and that they were not in contact with living bone. My human cases demonstrate the fact that large grafts without the periosteum may die, and I infer from this that the blood-supply to the large grafts is insufficient to keep them alive. Since, if the periosteum be left attached, the graft may not die, the soft and porous structure (as compared with bone) of the periosteum permits it to get its blood-supply from the surrounding tissues, and from this the bone is reformed.

Experiment 3 (Dog 363).—Two-thirds of each fibula were resected with their entire periosteum. Each fibula was split into two pieces, making four sections in all. From two of these pieces the periosteum was removed, while the other two were transplanted with their periosteum still attached. The external oblique of the left side was dissected up, just below the last rib, whose periosteum was split and separated from the bone. One of the four strips (without periosteum) was sutured to this bared area of the last rib. To the end of the first strip was sutured a second strip of fibula with periosteum on half its surface. To the end of the second piece of bone was sutured a third strip of fibula without periosteum on any of its surfaces, and to the end of the third piece was sutured the fourth strip of fibula with periosteum on half its surface.

Result.—The defects made in the fibulae have not been filled in with new bone in the slightest. 108 days after operation. The strip *A* (Fig. 2) without periosteum has been entirely absorbed. The strip *B* with periosteum remains perfectly alive and shows no signs of any absorption. The strip *C* without periosteum has become broken up into six pieces, which have become displaced upward. All these irregular pieces look alive. The strip *D* with periosteum is alive and its irregularities show that it has produced new bone.

This experiment demonstrates that without periosteum there is always uncertainty as to what a graft without it will ultimately do. In some cases the bone will remain living, in other instances it will become absorbed, in other cases it will break up into small pieces, all of which goes to show that the question of blood-supply is the all-important factor. With periosteum we are sure of the grafts living.

EXPERIMENT 4 (Dog 220).—Two inches were excised from two ribs with their entire periosteum on all sides. The last rib was bared, the external oblique split. The last rib was separated from its periosteum for a short distance, and to this bared area was sutured one end of one of the ribs with its periosteum on all sides intact. To the other end of the transplanted rib was sutured one end of the second rib with periosteum on all its sides, by drill-holes in the two extremities. The two ribs were then transplanted between the external and internal oblique muscles.

Result.—The defects in the two ribs have not in the slightest become filled in with new bone, 112 days after operation. This is probably due to the fact that there was no channel left between the ends. The transplant A (Fig. 3), has become fractured in two pieces, A^1 being the second and lower piece of A. Both these pieces are undoubtedly alive. The upper part of A has become displaced from the last rib. This is another demonstration of the fact that grafts do not need to be in contact with living bone in order to remain living, contrary to Dr. Murphy's teaching. The second grafted rib Bi (with periosteum) has likewise become fractured, B being the small upper part of Bi. The entire graft B likewise remains living, although it is not in contact with living bone.

EXPERIMENT 5 (Dog 423).—A section from the right fibula intact without periosteum was transplanted into a left fibula defect in contact with old stumps. The stumps of the right fibula defect were covered with muscle which was sutured over them. The entire section from the left fibula with the periosteum still on it uninjured, was placed under the fascia of the right leg.

Result.—Eighty-one days after the operation, the section into the left fibula defect without periosteum has almost completely disappeared (*A*, Fig. 4), There is a slight line left indicating a persistence of some of it. This disappearance has occurred notwithstanding that it was in contact at both ends with living bone. The difference the presence or absence of the periosteum makes is indicated in the right leg. The section from the left fibula into the right fibula defect with periosteum has remained of its original size and seems perfectly alive (*B*, Fig. 4), notwithstanding that it was not in contact

with living bone. This experiment is another evidence that Murphy is mistaken in his statement that grafts must be in contact with living bone in order to live. This experiment would also seem to indicate that the periosteum is the important element in maintaining the life of grafts, owing probably to its influencing favorably the blood-supply.

Experiment 6 (Dog 426).—Sections from each fibula were excised. From the section from the right side all the periosteum was bluntly stripped off from all its sides in one place. This strip of periosteum *C* was vertically stretched out under the skin of the left leg. The bone itself from the right fibula without periosteum, *A*, was placed in contact with the ends of the old stumps in the left fibula defect. Over the ends of the stumps of the right defect in the fibula, muscle was sutured so that the graft would not be in contact with living bone, and the section *B* from the left fibula with its periosteum entire was placed in the superficial muscles of the right leg. The section of bone with periosteum in right leg, *B*, not in contact with living bone, has grown to two or three times its normal size and is certainly perfectly alive.

Result.—One hundred and two days after operation (Fig. 5). The piece of fibula *A* without periosteum from right fibula into left defect has entirely disappeared. This is probably due to the fact that the bone was transplanted entire. Had it been split as in the first two experiments it is possible that it would have survived, owing to the consequent better blood-supply. The periosteum, *C*, on the other hand, transplanted under the skin of the left leg, has developed a new mass of bone of the size and shape of the old fibula from which it was taken.

Figures 6, 7 and 8 are roentgenograms of the same human transplantation, in which a defect in the tibia had to be supplied by a graft. In Figure 6 we see 5 inches of the opposite, healthy fibula transplanted into the medullary cavities of the stumps of the old tibia. The lower extremity of the graft was held in place by an encircling wire strand. Periosteum covered the graft on all its sides and this was unsplit. Figure 7 was taken seven months after the grafting. A fracture took place in the graft about two months after the operation, owing to the child kicking off the splint one night. In Figure 7 we see what an enormous callus, *B*, developed just above the fracture. I do not wish to be too dogmatic about where the new bone of the shaft *A* and that of the callus *B* come from, but I unhesitatingly think that this new bone is derived either from the periosteum itself or rise from the bone cells of the graft itself. Were it

derived from the ends *E* and *F* of the old bone of the upper and lower stumps, it seems to me that it would shade off the farther we went from the ends of the old stumps, being greatest in amount near the old bone and shading off to become less and less the farther we went from the stumps. As a matter of fact, the greatest amount of new bone is at the fracture spot in the graft, which is some distance from either end of the stumps. The lower fragment, *D*, of the graft has not developed proportionately, owing to the inhibitory influence of the wire strand, which is eroding the fragment. This wire was then removed. Figure 8 was taken three months after the removal of the wire strand, and ten months after the original grafting operation. The lower fragment, *D*, has developed greatly, the furrow made by the wire being more than completely filled in by new bone. The fracture is consolidating nicely. That the bone of the lower fragment, *D*, has a life independent of the lower stump, *F*, is indicated by the lower stump, *F*. It is clearly seen that *D* is uniting to the lower stump *F*, and also to the graft *B* by life inherent in itself. The callus, *B*, is much smaller in Figure 8 than in Figure 7.

EXPERIMENT 7 (Dog 293).—Figure 9 was taken nine months after the transplantation, in which a segment from the left fibula was divided into as small fragments as possible, with the periosteum left attached to each fragment, and these fragments were transplanted into the right fibula defect in contact with the stumps of the fibula. Into the left fibula defect were transplanted fragments from the right fibula from which all the periosteum was scraped.

Result.—The fragments into the left fibula defect *A* (without periosteum) have almost completely disappeared. The fragments are few in number, isolated, not united, and they look as though they were becoming absorbed, being much reduced in size. In contrast to these, the fragments transplanted into the right fibula defect (with periosteum), have in most places become united to each other, show no diminution in size and cause healthy shadows. It seems evident that there was not supplied enough blood to the left fragments without periosteum to keep them alive. This picture is another evidence of the value of the periosteum and is another proof of the fact that contact with living bone is not the important element which Dr. Murphy has attributed to it, and demonstrates the value of the periosteum.

EXPERIMENT 8 (Dog 302).—Fragments from a section of the right fibula from which all the periosteum was thoroughly scraped, were grafted into a defect made in the left fibula

Fragments with periosteum still attached to them, taken from a section of the left fibula, were transplanted into the right fibula defect.

Result After Eight Months.—The fragments into the left fibula defect without periosteum have entirely disappeared. The pieces into the right fibula defect with periosteum have markedly increased in size and have largely united to each other. This case illustrates the value of the periosteum on grafts in case there is a deficiency in the blood-supply. The feature to be particularly emphasized in this experiment is the pronounced increase in the size of the grafts with periosteum on them transplanted into the right defect, which denotes an active life in the grafts; also the fact that, although the middle section of the right-sided graft does not seem to be firmly united to the neighboring grafts, yet this middle section, from its deep shadow, appears to be as actively alive as the sections which are in intimate contact with the ends of the old stumps, all of which still further goes to show that contact with living old bone has not the important influence on the subsequent life of grafts which Dr. Murphy has attributed to it.

Experiment 9, Figure 10.—This animal was killed six months after the operation. The removal of a section of a rib with its attached periosteum shows no reformation of the rib. The periosteum was stripped off the section of rib bluntly and this sheet was planted between the rectus muscle of the abdomen and its posterior sheath.

Result.—On cross-section six months later, this transplanted periosteum cuts as hard as bone and with the same sensation (Fig. 10). A photomicrograph of a section of this new bone from the periosteum shows that there is a distinct mass of bone, measuring 0.8 by 0.2 cm., which is enclosed in a definite connective-tissue capsule. The bone itself contains cells, the nuclei of which stain deeply with hematoxylin. At points on the periphery of the bone there are osteoblasts. In the actual bone substance itself there are haversian canals which contain blood-vessels the size of large arterioles. Within the bone trabeculae there are adult fat- and marrow-cells, and the whole presents a normal appearance. There are many blood-spaces in the marrow and these spaces are supported by a definite connective-tissue stroma. The red blood-cells in these blood-spaces, while not sharp and distinct in outline, are not degenerated. There are no osteoclasts or round cells present, suggestive of either a destructive process or one of inflammation. The bone is alive and the periosteum has reformed a normal rib.

Experiment 10 (Dog 374).—From each fibula 2½ inches were resected. The periosteum was removed from each *in toto*. The periosteum from the right fibula section was a beautiful sheet, removed without scraping, by stripping it off the bone

bluntly. This was transplanted just under skin of the left leg, lying on the muscle. The periosteum from the left fibula section was a frayed-out strip which was scraped off with a knife. This was transplanted under the skin of the right leg. The right fibula section without periosteum was transplanted into the left fibula defect and the left fibula section without periosteum was transplanted into the right defect.

Result.—After 142 days each fibula section without periosteum has remained living perfectly. That transplanted periosteum may produce bone is here again demonstrated. The perfect sheet of periosteum transplanted into the left leg has produced a fine piece of bone, while the frayed-out piece of periosteum into the right leg has produced just a suspicion of new bone (Fig. 11). This indicates that the periosteum is a delicate structure. It is worthy of remark that the periosteum into the left leg was removed bluntly by tearing it off the bone and was not obtained by scraping it off with a knife, which procedure would carry off with the periosteum some of the superficial bone cells.

WILL TRANSPLANTED PERIOSTEUM PRODUCE NEW BONE?

There have been various answers to this question. Macewen has minimized the importance of the periosteum when he says that it acts merely as "a limiting and protecting membrane and has no osteogenetic function." He has not found that it produced new bone when it is raised up in strips from the bone. I have found that new bone would develop about transplanted periosteum in about one-quarter of the cases in which it was transplanted alone. Thus, in Figure 10, the periosteum from a rib, transplanted into the abdomen, has produced new bone. Also in Figure 5, a fine growth of bone, *C*, is seen to have developed about the transplanted periosteum. Likewise in Figure 11, new bone, *C*, has developed about the transplanted periosteum. In many cases I believe that the periosteum is so greatly damaged in separating it from the bone that it will not produce bone. To my mind the question of whether or not periosteum will produce new bone when it is transplanted alone into the soft parts is beside the mark and of little importance, because, as I believe, the function of the periosteum is to maintain the nutrition of the grafts quite aside from any osteogenetic function that it may have.

CONCLUSIONS

1. The theory that contact with living bone is necessary for the subsequent life of grafts must be given up.

2. Living bone-grafts have life inherent in themselves and are capable of permanent growth even when transplanted into the soft parts.

3. The life of grafts is dependent on a sufficient blood-supply. This is surely obtained when periosteum is on the grafts, since practically 100 per cent. of such grafts are successful, whether the transplants be in contact with living bone or not, or whether they be grafted into the soft parts and not in contact with living bone.

4. Forty-eight per cent. of my bone grafts without periosteum were successful whether contact with living bone was made or not. This goes to show that there is some other factor present making for the life of grafts than the periosteum or contact with living bone, and this I take to be a sufficient blood-supply. It is therefore evident that the periosteum has a deciding influence in favoring a good blood-supply with its consequent subsequent life of the grafts. Since it is impossible to tell beforehand when grafts without periosteum will attain this sufficient blood-supply, the lesson is evident that grafts should always be transplanted with as much of their attached periosteum as possible.

5. Periosteum transplanted into the soft parts will produce new bone in a certain proportion of cases.

32 East Fifty-Third Street.

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The Use of Herudin in the Transfusion of Blood

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THE USE OF HERUDIN IN THE TRANSFUSION OF BLOOD*

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The present communication is the last of three reports on experimental work undertaken to develop a more widely useful method of blood transfusion than those already existent. This work was inspired by the recent advances in the study of blood coagulation by W. H. Howell and other investigators of the Baltimore school, which suggested that the practical difficulties of developing a safe and easy transfusion method might be met on a more rational basis in the light of better knowledge; and that the newer laboratory methods of estimating the various factors of coagulation would be a help in forming a more precise judgment as to the efficacy or non-efficacy of measures calculated to prevent harmful changes in transfused blood.

The first part of our experimental work was concerned with influencing the initiative changes of coagulation through preventing or hindering thromboplastin formation by the use of paraffin and a suitable instrumental technic.[1]

The second part of this work resulted in the further development of a practical form of apparatus

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*The spelling used in this article for the name of the active principle of the secretion derived from the buccal glands of the pond-leech is that given to it by the discoverer, Friedrich Franz.—Ed.

1. Satterlee, H. S., and Hooker, R. S.: Experiments to Develop a More Widely Useful Method of Blood Transfusion, Arch. Int. Med., January, 1914, p. 81.

adapted to paraffin and herudin methods, and especially designed to enable a single operator to perform a transfusion, if necessary, without trained assistance.[2]

The third part of our investigations, concerning herudin, has been in progress along with the work already reported, but we have thought best to reserve the publication of this work until satisfied that the results warranted practical application.

Oxalated, citrated and fluorided plasmas are well known in the physiologic laboratories, and sodium citrate is reported to have been used as an anticoagulant for small quantities of transfused blood. Oxalate and citrate solutions act by fixing the calcium of the blood, which is a necessary factor in spontaneous coagulation. This decalcification is, of course, a change produced by a chemical reaction in the blood, and is theoretically, at least, undesirable. The use of herudin as an anticoagulant is not open to this objection.

Herudin is the active principle of a secretion derived from the buccal glands of the pond-leech, *Sanguesuga medicinalis,* and has been classed by Franz as a secondary albumose. Its physiologic properties are variously regarded by different investigators. Morawitz believes that it acts by neutralizing thrombin and prothrombin (thrombogen). Shittenhelm and Bodong believe that it neutralizes neither of these factors but reacts with some, as yet unknown substance, which is derived from the plasma. Mellanby concludes, from what appears to be substantial experimental evidence, that herudin contains an antibody for prothrombin and also a very energetic antibody for thromboplastin (kinase).

It may be concluded fairly from the available evidence that herudin has a decided effect on the prothrombin-antithrombin balance and that it has a neutralizing action on thromboplastin. This action is best illustrated diagrammatically.

The scheme of representation is based on Howell's theory of the mechanism of coagulation, and provides, we believe, an adequate explanation of the phenomena which have been observed. According to this theory

2. Satterlee and Hooker: The Further Development of an Apparatus for the Transfusion of Blood, Surg., Gynec. and Obst., 1914 [not yet published].

the potential factors of coagulation may be expressed as shown in the diagram of the circulating blood (Fig. 1 *A*). The fibrinogen and calcium occupy a neutral position but have a potential affinity for prothrombin. The antithrombin and prothrombin have an affinity for each other and are loosely combined. This combination constitutes a balance, the so-called antithrombin-prothrombin balance, which, in normal circulating blood, is not quite equal, because there is a slight preponderance of the antithrombin. If anything happens to upset this balance, so that the antithrombin side is weakened, then some of the prothrombin is released, and, in the presence of free calcium, becomes

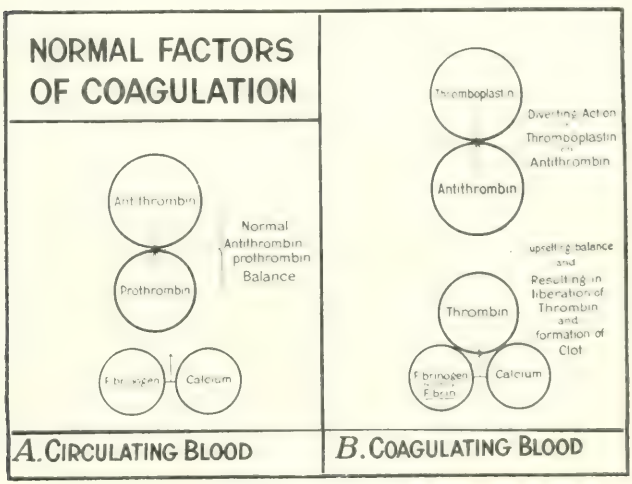

Figure 1.

thrombin; and this thrombin reacts with fibrinogen to form a clot.

This action is seen in Figure 1 *B*. Here the coagulative process is initiated by the advent of another element, thromboplastin, which, having a very marked affinity for antithrombin, diverts it from its prothrombin attachment, and clotting ensues in the manner indicated.

In Figure 2 it will be seen that the action of heruclin is to intervene and bind the thromboplastin, thus preventing the deviation of antithrombin from its prothrombin attachment.

There is considerable literature on the experimental use of herudin and there are some reports on its therapeutic use by intravenous injection for eclampsia, but no mention of its use as an anticoagulant for transfusing human blood.

From our experimental work it has become evident that herudin affords a convenient alternative for the paraffin method of transfusion under most circumstances. The amount of herudin necessary, with our apparatus, is so small that its use may not be contraindicated even in those pathologic conditions in which

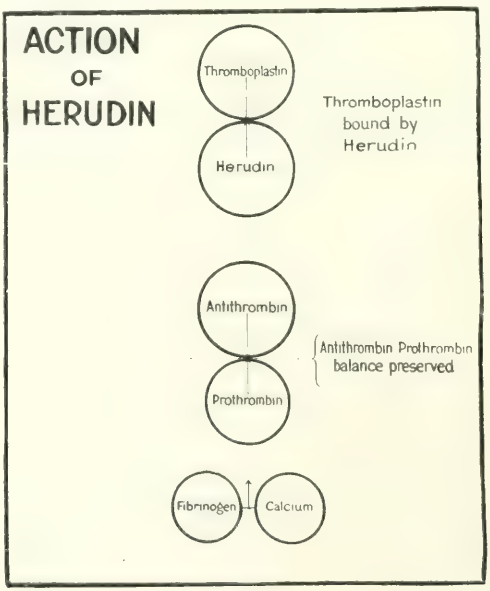

Figure 2.

there is already an excess of antithrombin or a deficiency of prothrombin in the circulating blood of the recipient.

Sievert has published a series of experiments which indicate that intravenous injections of herudin in rabbits are toxic in very large doses, that is from 25 to 50 mg. per kilogram of body-weight, the toxicity showing itself by changes in respiration and temperature, apathy, somnolence and albuminuria. Sievert states that the higher dosage, if quickly repeated, may

result fatally, and that necropsy reveals hyperemia and petechial hemorrhages of the kidney and spleen but no other lesions. Other observers have found comparatively little or no ill effect from the intravenous use of large doses of herudin. Kaposi has given 40 mg. of herudin in normal salt solution by intravenous injection to a 2-kg. rabbit without harming the animal, and designates this proportion, 20 mg. per kilogram of body-weight, as the normal dose for experimental work. Rimann and Wolf, using this dosage, particularly state that no disturbance of health was noted as a result. Cowie has given thirty-five intravenous injections of herudin to a rabbit in doses increasing from 10 mg. to 22 mg. in twenty-six days, and maintained the last-mentioned dosage until the final injection, the total period of treatment covering fifty-four days. At the end of this time the animal had gained in weight and was in every respect perfectly well. Bodong has given from 23 to 73.25 mg per kilogram of body-weight to rabbits, and states that it has no influence on the circulation or the respiration and is in no other way harmful to the animal. Von Hertzen and Ohman have confirmed Bodong's observation in a series of twelve experiments and concluded that herudin has no disturbing effect on the heart and blood-vessels. Abel, Rowntree and Turner have used very large quantities of herudin in "vividiffusion" experiments on dogs without apparently impairing the normal physiologic condition of the surviving animals. Dienst reports that he has given 200 mg. in 50 c.c. of salt solution by intravenous injection to a patient with very severe eclampsia, with most excellent results; and Engelmann has reported fourteen cases of eclampsia treated in this way with doses of from 200 to 300 mg.

Rimann and Wolf, in a series of eight experiments on the circulating blood of rabbits, have found that 20 mg. of herudin per hundred gm. of blood (normal dosis of Kaposi) renders the blood uncoagulable for four and a half hours, and that 10 mg. per hundred gm. of blood results in a shortening of the period of incoagulability to from one and a half to one and three-fourths hours. In these experiments the herudin was injected intravenously and the amount of circulating blood was calculated as one-tenth of the

body-weight. The evidence of a coagulative tendency in the circulating blood following the injection was obtained by introducing a thread of catgut within the blood-current, according to the method of Trendelenburg. This catgut was withdrawn at varying intervals and the earliest appearance of fibrin formation was noted.

EXPERIMENTAL BASIS OF THE METHOD

Our first aim in experimenting with herudin was to determine the minimal amount of this substance which, when used in conjunction with the best available technic, would serve to prevent coagulative changes for a sufficient length of time to insure a safe transfer of blood from donor to recipient. The first experiment of this series was designed to show the amount of herudin necessary to defer coagulation from one-half to three-quarters of an hour when mixed with blood which was withdrawn directly from the vein so that there was the least possible chance of contamination with traumatized tissue. In this experiment no paraffin was employed, the blood being taken from a vein in 1-c.c. glass pipets and transferred immediately to six glass tubes containing varying amounts of herudin in 0.9 per cent. sodium chlorid solution.

From this experiment it was estimated that about 3.5 mg. of herudin to 100 c.c. of blood would be requisite for purposes of transfusion without the aid of paraffin, provided good technic was employed in obtaining the blood free from admixture of tissue juices.

This, in actual trial with our regular apparatus, was found to be the case; but the time of onset of coagulation varied within fairly wide limits — from seven to fifteen minutes.

When a paraffin coating was applied to the tip and the neck of our transfusion pipet it was found that the amount of herudin could be reduced by half, or to 1.5 to 1.8 mg. per hundred c.c. of blood, and that under these conditions there was also a twofold lengthening of the time of onset of coagulation. This meant a fourfold increase of efficiency of the herudin in the presence of a paraffin lining of the tip of the transfusion pipet.

As compared with a 30-c.c. piston-syringe with cannula 1 mm. in diameter and 30 mm. in length, 4.0 mg. per hundred c.c. of blood gave a clotting-time of ten minutes and thirty seconds for the syringe method, whereas 1.5 mg. per hundred c.c. of blood gave a clotting-time of thirty minutes for the paraffin-sealed pipet; and this, by a simple computation, shows a relative efficiency of approximately 1:8 in favor of the pipet.

EXPERIMENTAL AND CLINICAL TRANSFUSION WITH HERUDIN

Case and Experiment No.	Amt. of Blood Transfused c.c.	Amount of Herudin Used mg.	Dilution of Herudin with NaCl Sol.	Time of Onset of Coagulation, Minutes	Time of Complete Coagulation, Minutes
*Exp. Transfusion 5	100	3	1 1,000	5	15
*Exp. Transfusion 6	150	3	1 1,000	8	13
Exp. Transfusion 7	100	3	1 1,000	40	†
Exp. Transfusion 9	150	3	1 1,000	†	†
Exp. Transfusion 11	200	3	1 [illegible]	[illegible]	35
Clin. Case 1:					
Cylinder A	200	3	1/500	20	†
Cylinder B	200	3	1/500	16	†
Clin. Case 2:					
Cylinder A	220	3.4	1/450	35	†
Cylinder B	220	3.4	1/450	30	†
Exp. Transfusion 15	160	3	1/500	21	†
Exp. Transfusion 17	150	3	1/500	[illegible]	35
Exp. Transfusion 18	220	3	1/500	35	38‡
Exp. Transfusion 19	220	3	1/500	30	38

* In these experiments the tip of the pipet was not lined with paraffin.
† Not observed.
‡ No clot at 38.

To demonstrate the effect of herudin as applied to our method of transfusion, we have appended a table including all of our experimental and clinical transfusions with herudin in which no special factors were introduced (as varying pressures in delivery of blood, etc.), which would influence the onset of coagulation in the blood which was reserved in the pipet for observation. Experimental Transfusions 5 and 6, in which no paraffin was used as a lining for the tip of the pipet, are included in this table for the special purpose of comparison with our preferred method, in which the tip of the pipet is paraffin-sealed and lined.

PRACTICAL APPLICATION OF THE METHOD

In making use of herudin for transfusion we have employed our regular pipets and cannulas, but we have dispensed with the paraffin coating of the cylinders. We have prepared the pipets by introducing from 3 to 5 c.c. of a 1:500 solution of herudin in salt solution, flowing this liquid very thoroughly over the interior of the cylinder and draining away the excess through the tip of the pipet just previous to use. From 1.5 to 2 c.c. of this solution, or approximately 3 mg. of herudin, are retained by adherence to the walls of the pipet, and for more than twenty minutes this is sufficient to prevent 220 c.c. of blood, drawn into the pipet, from clotting.

With the exceptions already noted, the operation with herudin is conducted in precisely the same way as with the paraffin-coated apparatus. The preparation of the pipets is the same, except that the first coating of the cylinders with paraffin is omitted. A partial coating with paraffin, however, is advisable. This coating should be done by aspirating the sterile, melted paraffin mixture just within the neck of the cylinder and expelling it again. This use of paraffin, from the tip to the neck, is primarily to insure an air-tight junction of the pipet tip with the metal bushing, and of the latter with the neck of the cylinder, but it also has a demonstrable effect in lessening thromboplastin formation during the aspiration of blood, and permits the employment of a minimal quantity of herudin. The herudin method may be employed without any paraffin whatever by using a 1:300 solution of herudin for coating and with care that the interior of the pipet tip is perfectly smooth and brightly polished, and that all connections are tight against air-leakage; but these contingencies are so surely and easily provided for by aspirating a little sterile paraffin just above the neck of the pipet, that the latter method is to be preferred. Any small receptacle with a cover and alcohol heating device will answer the purpose for sterilizing and holding the paraffin. The hardened paraffin may thus be carried with the apparatus, and the sealing process may be done at the time of operation, allowing ten or fifteen minutes for the pipet to cool, before coating with herudin solution.

It would, no doubt, be possible to obtain a more accurate estimate of the coagulation factors in transfused blood, and also of the inhibiting effect of herudin, by quantitative determinations of thrombin and of thromboplastin in specimens of such blood under varying conditions. We believe, however, that the simple observation of onset of coagulation together with the amounts of blood and of herudin, as recorded in the table, justify our conclusions. We hope at some future time to follow our present work with a more complete investigation of the subject with the aid of more precise laboratory methods.

We have appended a selected list of the references since 1905 which we believe to be of most importance and interest in connection with the subject. The literature prior to 1905 is well covered in the bibliography of Morawitz published in that year and we have included no earlier references except those which have been mentioned in the text.

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THE PROCESS OF BONE REPAIR FOLLOWING TRAUMA.

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The process of the repair of bone after trauma seems to be more or less shrouded in mystery. In reviewing the literature one finds the greatest diversity of opinion. This is largely to be attributed to two facts: (1) that clinicians have reported results frequently without consulting the microscopic findings, and (2) that satisfactory microscopic examinations are difficult because of the injury incurred by the soft parts while the bone is undergoing decalcification. Some authors invest the periosteum with bone-reproducing qualities, while others claim that it is nothing but a limiting membrane.

In the embryo there are two types of primary ossification, intramembranous and intracartilaginous.

Intramembranous Ossification. The basis for development is embryonal connective tissue or mesenchyme. At certain points these embryonal connective-tissue bundles become impregnated with calcium salts, the cells in each of these areas increase in number, some of them becoming more or less round or oval, with distinct nuclei and a considerable amount of cytoplasm, and arrange themselves in single, fairly regular rows along the bundles of calcified fibers. These differentiated cells are known as osteogenetic cells or osteoblasts. Under the influence of the osteoblasts a thin layer of calcium salts is deposited between the osteoblasts and the calcified fibers forming true bone. Successive layers of calcium salts are laid down, enclosing some of the osteoblasts, which then become bone cells. The spaces in which the bone cells lie are called lacunæ.

Intracartilaginous Ossification. In this type of ossification the bone is preformed from hyaline cartilage, which is also derived from embryonal connective tissue. Around this cartilage is a fibrous connective-tissue membrane called the perichondrium. This membrane gradually fades on the one side into cartilage and on the other into the surrounding connective tissue. There is no sharp

dividing line. During the early stage, calcium salts are deposited in the matrix of the cartilage, forming an "ossification centre." Later small bloodvessels push their way in from the perichondrium of the new diaphysis, carrying with them some of the embryonal connective tissue. These ingrowths of bloodvessels and connective tissue are called periosteal buds. The septa between the cartilage cells break down, forming large spaces into which the periosteal buds grow. Many of the connective-tissue cells are transformed into osteoblasts, and with the fibers and bloodvessels constitute osteogenetic tissue. This process continues until the primary spongy bone is formed, this being later destroyed by osteoclasts, with a subsequent formation of compact bone.

It is evident from this brief *resume*, that bone in the embryo is formed by a metaplasia from the connective tissue, accompanied by a deposition of calcium salts. Of interest in this connection is the work of Gideon Wells, who has shown that if cartilage is transplanted into the omentum it has a greater power of absorbing calcium from the blood than any other tissue similarly placed.

Theories in Regard to Bone Repair. 1. *Periosteum the Main Agent in Repair.* This has been recently the most generally accepted theory. The adherents of this theory consider that the periosteum is a specialized tissue for the formation of new bone, and that bone deprived of its periosteum will eventually die. Much experimental work has been done along these lines. Numerous investigators have transplanted periosteum and observed new growth of bone. MacEwen has offered the criticism that in removing the periosteum a layer of bone cells, osteoblasts, are transplanted also. If a piece of the periosteum, to be transplanted, were saved for microscopic examination this question could be satisfactorily solved. Morpugo demonstrated that the periosteum of a corpse would reproduce new bone when implanted after one hundred and sixty-eight hours. Murphy thinks that if periosteum be transplanted in the same individual, especially if he be young, it will form a lasting bone deposit; but if transplanted into another individual, it rarely produces bone. He, however, states that bone transplanted with or without its periosteum into fat or muscle tissue eventually dies. MacWilliams, in a paper read before the American Medical Association, states that periosteum is necessary for the life of bone transplants, unless the transplants are very small, in which case they grow and unite without periosteal aid. He has demonstrated in his experimental work that large bone fragments transplanted into the soft parts will live if the periosteum be attached, but that they are almost constantly absorbed when transplanted without the periosteum.

2. *Periosteum not a Bone-forming Organ.* MacEwen considers the periosteum to be merely a limiting membrane without osteogenetic functions. He has performed numerous experiments which

seem very convincing, although it is frequently difficult to understand his deductions. He has, for instance, removed about 1 cm. of the radius, scraped off the periosteum, and cut the piece removed into small fragments, which were reinserted between the cut ends of the radius. Eleven weeks later the animal was killed and the leg examined. To quote from MacEwen: "Small diaphyseal grafts, when placed in a gap in the continuity of bone, show active proliferation from the whole circumference, each piece being an ossifying centre from which osseous tissue is thrown out sufficient to fill the gap between the various fragments and to unite them together along the two ends of the divided bone."

MacEwen and MacWilliams have both shown that small fragments of bone transplanted into the soft parts will unite and form definite bone, although MacEwen has not allowed time enough to see if the bone would eventually be absorbed.

In transplanting sections of the diaphysis, most authors are united in thinking that the transplanted piece dies, but that union occurs by new bone growing in from the shaft of the host, and also by new bone forming in the Haversian canals and medulla of the transplanted piece.

Barth can see degeneration in the nuclei of the transplanted fragment in one week; in young animals he sees small groups of bone cells until the twenty-second day.

Axhausen has transplanted joints and bones into soft parts, and finds three stages in the transplanted piece. (1) the nuclei take a normal stain; (2) the nuclei become crumpled and distorted; (3) the nuclei disappear and the bone becomes homogenous.

On the other hand, Takata finds that isolated bone fragments are surrounded by irregularly lying, dentated, relatively large cells that closely resemble osteoblasts. These cells are not separated from the surrounding connective tissue, and they lie without any sharp differentiation. The connective-tissue cells become gradually larger as they approach the bone. He considers these fragments to be living bone.

Of great interest is the formation of bone in arteries, new-growths, etc. Bunting, Harvey, Wells, and others have described the formation of true bone in arteries. In Bunting's case there was a formation of bone in a sclerotic aorta. This tissue had a definite lamellar structure with Haversian canals, and a narrow cavity, containing the normal cellular elements. He made a careful histological study, and his conclusions were as follows: "Bone in arteries may be desposited in two ways; either by the conversion of connective tissue into a callous or osteoid tissue, with subsequent conversion into bone and at times with the formation of cartilage, or by the resorption of calcified tissue by vessels and osteoblastic membrane, derived by metaplasia from the connective-tissue cells accompanying the vessel."

Wells believes the reason bone is so rarely deposited in veins is that the excess of carbon dioxide in the venous blood keeps the calcium in solution.

Harvey gives the following theories for the formation of bone in arteries: (1) wandering osteoblasts; (2) metaplasia of connective tissue; (3) metaplasia of mesothelium of blood capillaries. He produced new bone in the aorta of rabbits by painting the adventitia with 3 per cent. solution of silver nitrate, or 2 per cent. solution of copper sulphate. He killed them after various lengths of time, varying from one hundred and ten to one hundred and eighty-six days, and found calcium plaques along the inner edge of the media. On sectioning these plaques he found definite lamellar bone, with Haversian canals, lacunæ, and red marrow cells.

Liek, Poscharisсky, Sacerdoti, and Frattin have tied off in rabbits the renal vessels on one side. In from sixteen days to two months the animals were killed. True bone was found adjacent to the calcium deposits in the necrotic connective tissue of the kidney; this was accompanied by a growth of granulation tissue inward from the capsule. Here also was definite lamellar bone.

The writer's work was undertaken to attempt to learn the method of repair in bone cavities. To accomplish this, Dr. Clarke, director of the surgical research laboratory of Columbia University, suggested making simple cavities in the diaphysis of the humerus in dogs. An effort was made to secure a cavity in which absolute hemostasis occurred, and therefore a dead space. The humerus was exposed, usually by a median incision, the periosteum was removed for a distance of at least 3 cm. in length and for almost the entire circumference of the bone. A rectangular piece, 1 x 0.6 cm., extending into the medullary canal, was removed from the median surface of the diaphysis, and the medullary cavity was thoroughly curetted and packed until hemostasis occurred. Realizing that this hemostasis was only temporary, and that oozing probably occurred from the medullary cavity after suturing the muscles over it, various experiments were tried: (1) the cavity was packed and then filled with hot sterile oil and the wound closed; (2) the cavity was packed with gauze, which was removed in twenty-four hours; (3) the cavity was packed with gauze sterilized in oil, which was removed twenty-four hours later (the purpose of this was to prevent the gauze adhering to the sides of the bone cavity and the subsequent oozing upon its removal); (4) both ends of the cavity were filled with Morehof's bone wax; this caused very good hemostasis, and was used in the largest number of experiments.

In one case a sac was made of the peritoneum covering the appendix. The peritoneum was stripped off and a purse-string suture applied around the opening of the peritoneum; the sac was then filled with salt solution and placed in the bone cavity, completely filling it. The idea was to prevent the cavity from filling

with blood clot, and with the hope that the sac would be eventually absorbed. Unfortunately this dog received a gas bacillus infection and died in forty-eight hours.

After varying lengths of time the dogs were killed and the legs hardened in formalin. The gross appearance was then noted. Longitudinal or cross-sections were made. After decalcifying they were sectioned and examined microscopically. It has been a difficult thing to sufficiently decalcify the bone without destroying the soft tissue.

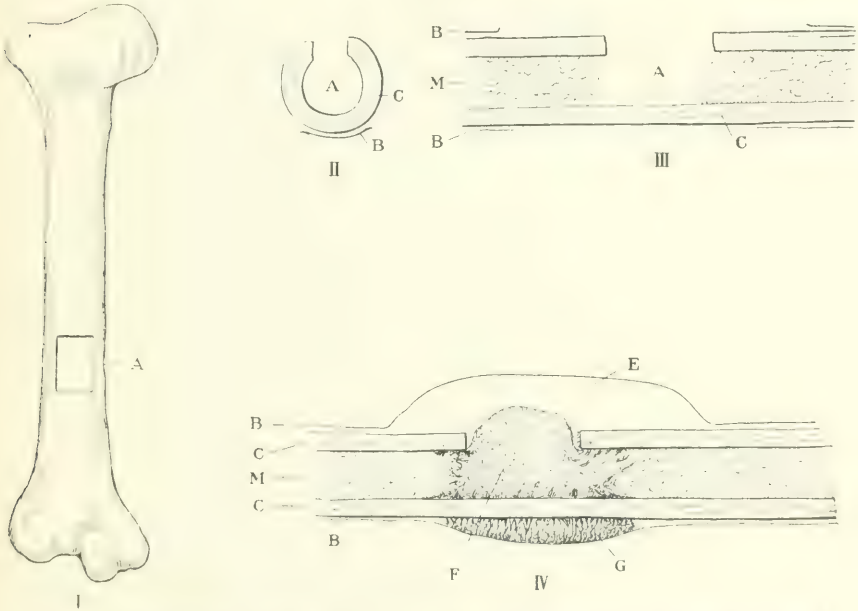

FIG. 1.—Schematic drawings. *I*, cavity in shaft; *II*, transverse section through bone and cavity; *III*, longitudinal section; *IV*, manner of repair of bone cavity. *A*, cavity; *B*, periosteum; *C*, shaft; *E*, cavity under periosteum filled with serosanguineous fluid; *F*, new-forming bone; *G*, bone forming under periosteum; *M*, medullary cavity.

RESULTS. Thirty-eight dogs have been operated upon as described above. Of this number, 4 died of acute infection, 4 sustained fractures following operation, and 6 died of either distemper or lobar pneumonia before a satisfactory length of time had elapsed. The remaining dogs were killed at varying lengths of time, ranging from thirty to one hundred and ninety days. A certain definite type of repair occurred in most of these cases. On cutting down upon the humerus a cavity containing a serosanguinous fluid was entered. The base of this cavity was bare bone of the diaphysis, while the periphery consisted of a flat connective-tissue layer that was continuous with the overlying muscles. These cavities varied from 2 to 5 cm. in length, and in some cases extended around almost the

entire circumference of the bone. In the bare bone at the base of this sac was found the former bone cavity extending into the medullary canal. Extending upward from the bottom of the cavity was a pyramidal mass of tissue which microscopically resemble granulation tissue. On microscopic examination this tissue was found to contain a ground-work of granulation tissue, with deposits of calcium salts arranged around the perivascular areas.

In a few cases a definite connective-tissue layer, continuous with the periosteum and adherent to the surrounding structure, has grown over the bone and the cavity has been repaired under this.

The four cases of fracture were sectioned and studied. Here there was seen a gradual transition of connective tissue into bone It was impossible to make out any definite periosteum.

In giving detailed accounts of experiments only those are given where it has been possible to get good photomicrographs. Those not mentioned have all been examined microscopically, and all show a similar process of repair to those given in detail. It seemed wise to omit giving their histories in detail on account of taking up too much space.

Dog No. 362. Surgical Research Series, 1912–1913. Experiment No. 21. Surgical Pathology, No. 2066. Operation, May 27. Death, due to pneumonia, June 8. Duration, twelve days. Median incision, 6 cm. long, over left upper foreleg. Humerus exposed. Periosteum removed for a distance of 3.5 cm. A rectangular piece of bone, 0.8 x 1 cm., was removed from the shaft. This incision extended into the medullary canal, which was curetted and packed for several minutes. Upon removal of the packing, good hemostasis was observed. The muscles were sutured over the defect in the shaft, and the skin was closed with continuous silk.

Autopsy. Lobar pneumonia of right upper lobes. Incision wound on leg perfectly healed. Leg hardened in formalin. Upon cutting down on the humerus a small sac, about 2 cm. long by 1.5 cm. wide, is opened. The base of this sac is the humerus and the apex consists of a smooth fibrous tissue membrane apparently continuous with the overlying muscles. This sac contains a serosanguinous fluid. In the humerus at the base of this sac is the aperture in the shaft, which is filled with blood clot.

Microscopic Examination (Figs. 1 to 3). A longitudinal section was made through the humerus. The bone was decalcified with phloroglucin and nitric acid solution. Plate I shows a schematic drawing of the operation and longitudinal sections taken for microscopic examination. The periosteum does not approach the aperture in the shaft for at least a distance of 1 cm. in all directions. Growing up from the base of the medullary canal and from its upper limits is seen a granulation tissue staining a deep blue. This tissue is made up of fibroblasts and other connective-tissue cells. One sees a net-work of tissue taking a purple stain which, under high

power, is seen to be bone. One is impressed with the idea that calcium salts have been laid down upon ordinary granulation tissue in the perivascular areas; that is, in the areas farthest from the bloodvessels. This is an early formation of new bone.

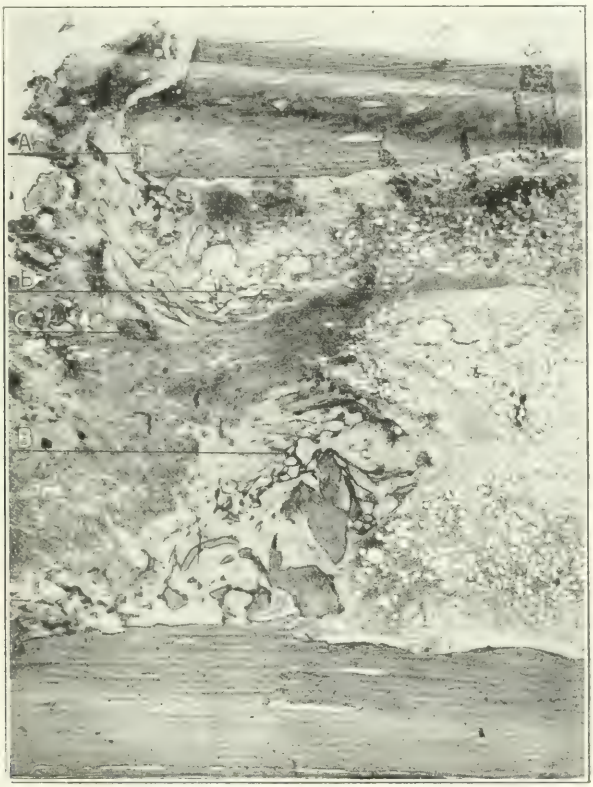

FIG. 2.—Surgical pathology, No. 2066. Longitudinal section through one-half of cavity Duration, twelve days. *A*, edge of bone cavity; *B*, new forming bone; *C*, blood clot.

Dog No. 127. Surgical Research Series, 1912–1913. Experiment No. 29. Surgical Pathology, No. 2237. Operation, December 3, 1912. Died December 19, of pneumonia. Duration, fifteen days. Operative procedure similar to Pathology No. 2066. Healing of operative wound *per primam*. Longitudinal sections made in routine manner. Upon gross inspection the periosteum found separated from the bone about 0.5 cm. on all sides of aperture in humerus. This bone cavity is seen filled with blood clot.

Microscopic Examination (Fig. 4). The centre of the cavity is seen to be filled with blood clot, and fine interlacing strands of new bone are being formed from the connective tissue. Under high power of the new bone one sees numerous round cells, with dark staining eccentric nuclei. It is possible that these cells are

either converting the earlier bone into a denser bone, or they may be phagocytes.

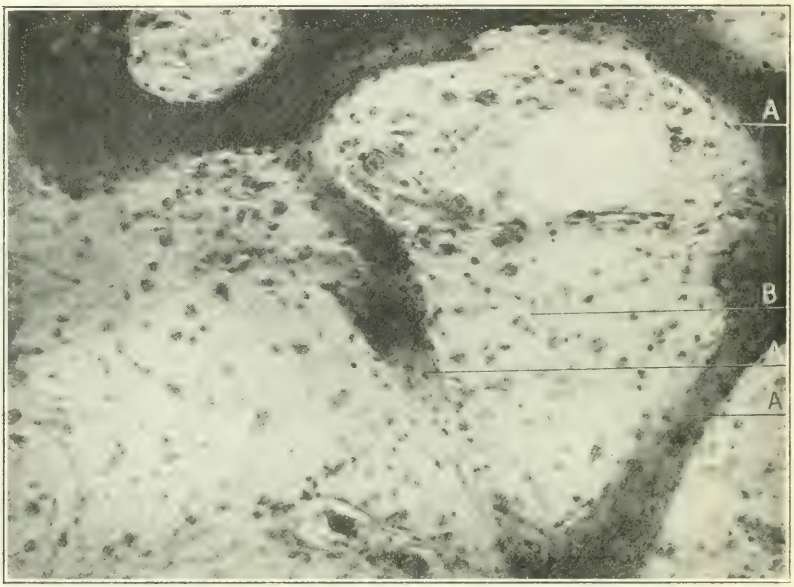

FIG. 3.—High power of part of Fig. 2. A new-forming bone, stellate-shaped cells; *B*, areolar connective tissue.

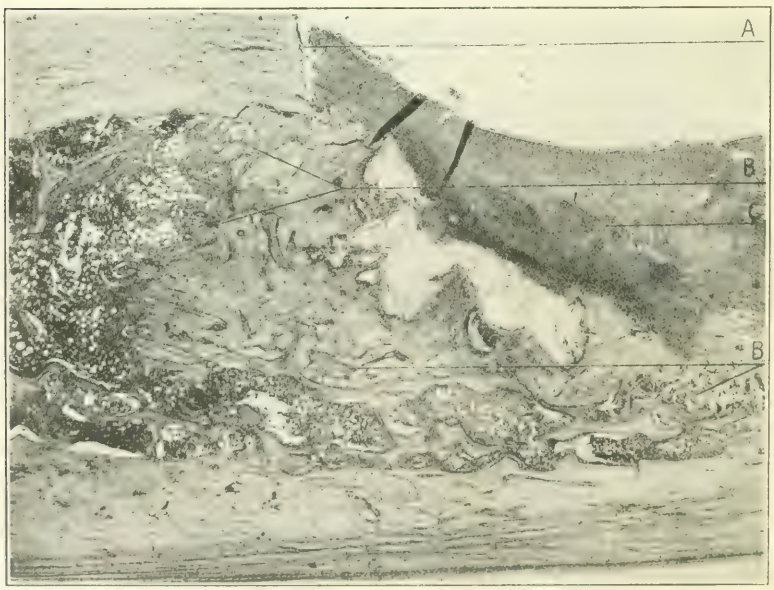

FIG. 4.—Surgical pathology, No. 2237. One-half bone cavity. Duration, fifteen days. *A*, edge of bone cavity; *B*, new bone; *C*, blood clot.

Dog No. 108. Surgical Research Series, 1911–1912. Experiment No. 4. Surgical Pathology, No. 1882. Operation, January 5, 1912. Died of pneumonia February 14, 1912. Duration, twenty-four days. Routine operation.

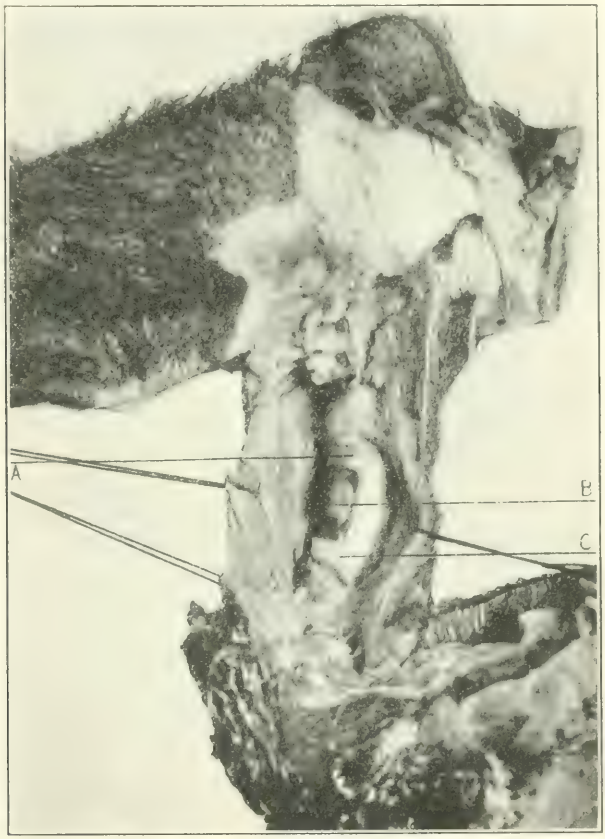

FIG. 5. Surgical pathology, No. 1882. *A*, edge of periosteum; *B*, new bone filling cavity; *C*, bare bone.

Autopsy. Lobar pneumonia. Operative wound healed *per primam*. Upon cutting down upon the humerus a sac containing a serosanguinous fluid was opened. This fluid, upon microscopic examination, contains numerous erythrocytes, polymorphonuclear leukocytes, and lymphocytes. The base of this sac is bare bone; in the centre is the aperture in the humerus. The apex of the sac is a smooth, connective-tissue membrane continuous with the overlying muscles. The periosteum is well separated from the bone cavity. The centre of this aperture is filled with a growth of new tissue resembling granulation tissue in appearance; on palpating

this with a needle it is hard and gritty. On longitudinal section this tissue filling the bone cavity is seen to be an early formation of new bone.

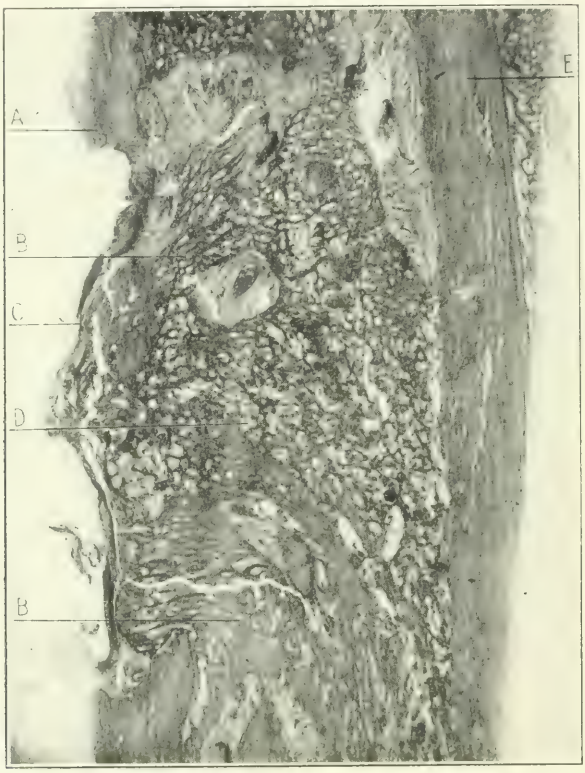

FIG. 6.—Low power. Longitudinal section of Fig. 5. *A*, edge of bone cavity, living bone; *B*, new bone; *C*, connective tissue; *D*, bloodvessels and areolar tissue.

Microscopic Examination (Figs. 5 and 6). This bone-granulation tissue arises from the base of the cavity; it has a honey-combed appearance, the lighter staining areas consisting of bloodvessels and connective-tissue stroma, while the darker stained areas are newly forming bone. Here under high power one cannot see that any specific cell forms this bone; it appears rather that calcium salts have been deposited in the perivascular areas upon connective tissue.

Dog No. 361. Surgical Research Series, 1911–1912. Experiment No. 20. Surgical Pathology, No. 2147. Operation, May 27, 1912. Killed October 15, 1912. Duration, 141 days. Piece, 1 x 0.5 cm., removed from lateral surface of left humerus; cavity curetted thoroughly; medullary canal at both ends filled with bone

wax. Excellent hemostasis. Muscles and skin sutured in usual manner.

Autopsy. Only slight linear operative scar visible; on palpation the bone feels smooth; no depression or nodules can be felt. On cutting down upon the bone a cavity, 5.5 cm. long by 1.5 cm. wide, is opened; it extends from immediately above the condyle to 1 cm. below the greater trochanter. This cavity contains a small

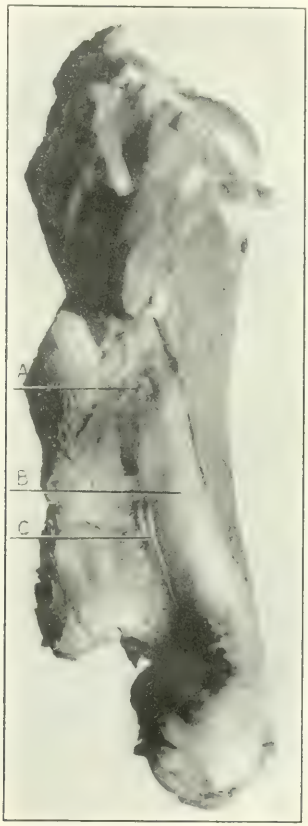

FIG. 7.—Surgical pathology, No. 2147. Duration, one hundred and forty-one days. *A*, bone cavity; *B*, bare bone; *C*, periosteum.

amount of serosanguinous fluid; its base is formed by bare bone. The centre surface is a fibrous tissue membrane adherent to and apparently continuous with the adjacent muscles. On the humerus about 0.5 cm. below the upper limit of this sac is a small oval depression in the bone; it measures about 4 mm. in diameter and 2 mm. in depth. At the bottom of this depression is a small shot-like piece of bone which has the appearance of a sequestrum; *x*-rays,

however, show there is no sequestrum, but there is a definite rarefaction of the bone at this location.

Microscopic Examination (Figs. 7 and 8). The bone which bridges over the aperture is much more cancellous than normal; the Haversian canals occur more frequently, and are larger. The bone cells are present and stain with hemotoxylin and eosin. In the small piece at the bottom of the depression the cells appear shrunken and stain poorly, but it has not the appearance of dead bone, as the cells and Haversian canal systems can be clearly made

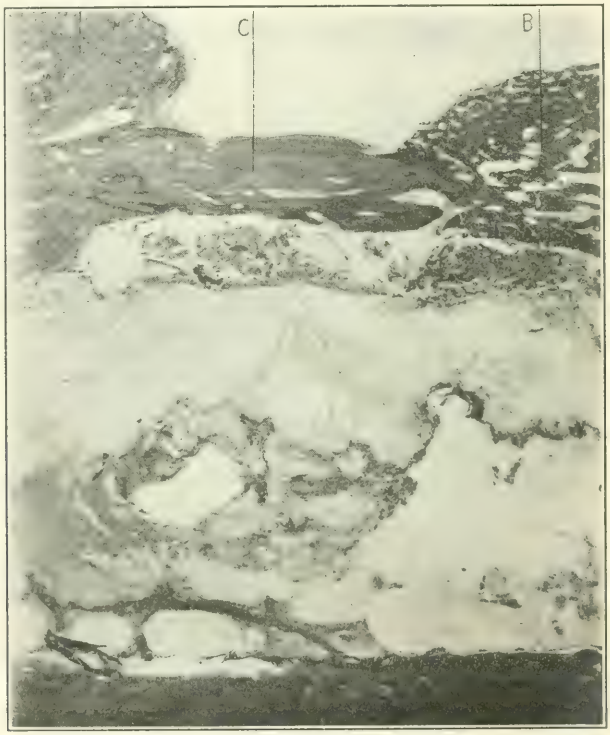

FIG. 8.—Low power of Fig. 7. *A*, new-forming bone; *B*, new-forming bone, showing enlarged Haversian canals; *C*, base of cavity *A* in Fig. 7.

out. Immediately below this bridge, in the medullary canal, is a cavity, which before decalcification was filled with bone wax and blood-clot; around this is a thin capsule of new bone. In this case the bone has reformed, with the periosteum stripped off from the bone for a distance of 5 cm. This bone is living bone; the process of repair is probably a later stage to the previous cases.

Dog No. 392. Surgical Research Series, 1911–1912. Experiment No. 24. Surgical Pathology, No. 2148. Operation, June 17, 1912. Killed October 15, 1912. Duration, one hundred and

twenty days. A piece, 1 x 0.8 cm., removed from the median surface of right humerus; the medullary canal was thoroughly curetted and packed; after removing the packing good hemostasis was observed. Muscles and skin sutured in usual manner.

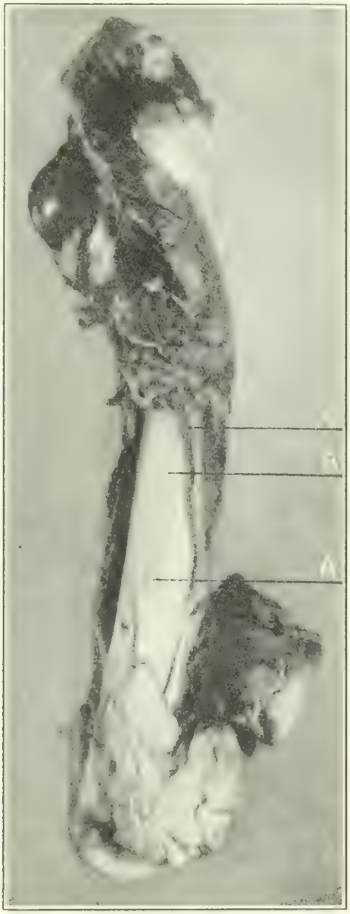

FIG. 9.—Surgical pathology, No. 2148. Duration, one hundred and twenty days. *A*, depression at situation of former bone cavity; *B*, bare bone; *C*, periosteum.

Autopsy. Only a linear scar visible; humerus feels smooth. On cutting down upon the bone a sac similar to Pathology No. 2147 is opened. It is difficult to observe where the aperture was in the humerus; there is a very slight depression in the bone.

Microscopic Examination (Figs. 9 and 10). The most noticable thing in the new bone is the direction of the Haversian canals. Instead of running parallel to the surface they lie perpendicular

to it, so that it is easy to determine the junction of old and new bone. In the medullary cavity numerous strands of bone are seen running toward the new bone. In comparing this section with Pathological No. 1882 it would seem that this is a later stage of a similar type of repair; the direction of the Haversian canals indicate that it originated from the tissue of the medullary canal.

FIG. 10.—Longitudinal section of Fig. 9, under low power. *A*, edge of former cavity—Haversian canals run perpendicular to surface; *B*, bone strands; *C*, bone at bottom of cavity.

Dog No. 325. Surgical Research Series 1911–1912. Pathological, No. 2180. Operation, May 6, 1912. Died December 7, 1912. Duration, one hundred and ninety days. Cavity made in humerus in usual manner, good hemostasis. Wound healed *per primam*. Animal in good shape; killed by chloroform.

Autopsy. On cutting down upon humerus it is difficult to find the site of the former operation, only a slight depression being visible. The bone is completely covered with fibrous tissue continuous with periosteum and adherent to the overlying muscles.

Microscopic Examination (Fig. 11). The aperture in the shaft is almost entirely filled with new bone. In this portion, however, the Haversian canals run perpendicular to the surface rather

than horizontal to it. Spicules of new bone are seen traversing through the tissue of the medullary canal immediately beneath the former aperture.

FIG. 11.—Surgical pathology, No. 2180, under low power. Duration, one hundred and ninety days. *A*, section of former bone cavity—Haversian canals run perpendicular to surface; *B*, junction of old and new bone; *C*, bone spicules in medullary cavity; *D*, bare bone.

Dog No. 88. Surgical Research Series, 1911–1912. Experiment No. 2. Surgical Pathology, No. 1907. Operation, January 12, 1912. Cavity in humerus by usual method. On January 24, the dog jumped off a chair and fractured the foreleg. Animal killed with chloroform February 21. Duration following fracture is twenty-eight days.

Autopsy. Wound in leg healed; there is non-union, with considerable over-riding of the fragments. There is considerable thickening about the ends of the bone. On cutting down upon the humerus one finds marked callous formation.

Microscopic Examination (Fig. 12). There is new bone proliferating from the outer surface of the ends of the fractured bone;

no definite periosteum can be made out. Under high power one finds a definite metaplasia from connective tissue to fibrocartilage, from connective tissue to bone, and from fibrocartilage to bone. In the areas of new-bone formation one finds first a layer of fibrous connective tissue that gradually changes into newly forming bone. There are all stages of metaplasia between the cartilage and the bone cells. The nuclei becomes irregular, often stellate, the deep blue of the capsule gradually fades and eventually disappears, and the cell takes on all the characteristics of the bone cell.

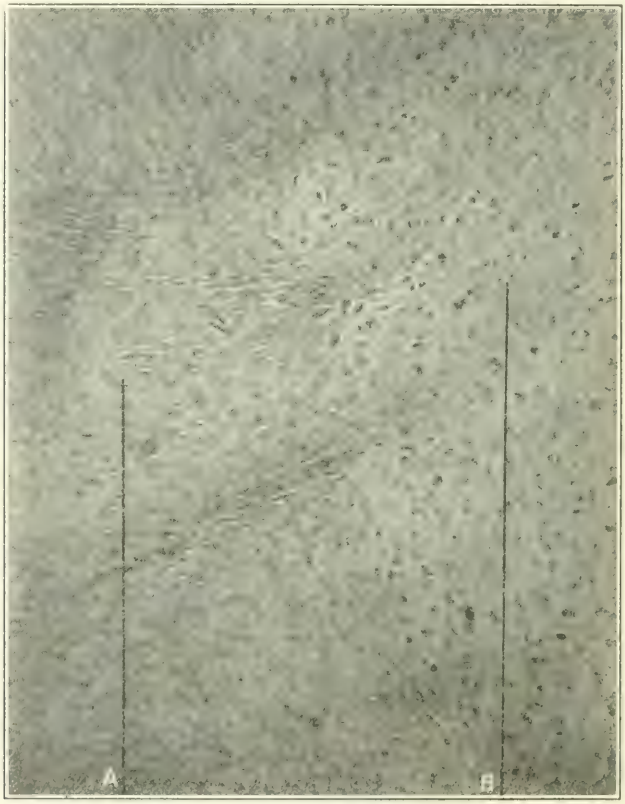

FIG. 12.—Surgical pathology, No. 1907, under high power. Transition of connective tissue into fibrocartilage. *A*, fibrous connective tissue; *B*, fibrocartilage.

Dog No. 251. Surgical Research Series, 1911–1912. Experiment No. 12. Surgical Pathology, No. 1997. Operation, March 25, 1912. Routine operation. On April 1 the dog jumped off a table and broke his leg. April 8 an attempt was made to get some callous cells to grow *in vitro*, and the leg was amputated at the fracture line. The transplants did not show any growth; a small

spicule of bone with surrounding callous was taken for microscopic section.

Microscopic Examination (Fig. 13). Under low power the specimen looks like a lace curtain, the darker perivascular areas being the calcium deposits while the lighter areas are granulation tissue and bloodvessels. This arrangement is clearly shown under high power.

FIG. 13.—High power of surgical pathology, No. 1997. *A*, callous (new-forming bone); *B*, bloodvessels.

Dog No. 117. Surgical Research Series, 1912–1913. Experiment No. 28. Surgical Pathology, No. 2250. Operation, November 25, 1912. Cavity made in usual manner. Wound healed by first intention. On December 10 the dog jumped off a chair and fractured his humerus. Animal killed with chloroform on December 27. Duration, seventeen days.

Autopsy. There is considerable callous formation around the broken ends. No union has occurred, as there is a definite false point of motion, with over-riding of the fragments. Leg hardened

in formalin. Cross-sections taken through broken ends of the bone, including callus and surrounding connective tissue.

Microscopic Examination (Fig. 14). Around the periphery of the shaft there is proliferating new-bone formation. It is impossible to make out periosteum, as it fuses with the general connective-tissue infiltration. Surrounding the callus and new-bone formation is a fibrous tissue stroma. The loose ends of the bone are covered with tissue that resembles cartilage.

FIG. 14.—Surgical pathology. No. 2250. Repair of fracture. *A*, connective tissue; *B*, transition of connective tissue to osteogenetic cells; *C*, new bone; *D*, cartilage cells embedded in bone.

Beginning at the periphery there is a gradual transition from the surrounding connective tissue into fibrocartilage. Beyond the cartilage one sees strands of new-bone formation; there are places in this new bone where there are islands of cartilage cells; the cartilage cells become gradually smaller; their nuclei becomes irregular or stellate in shape, and their capsules gradually lose their blue stain and disappear, until there seems to be no definite dividing line between the cartilage and bone cells.

Dog No. 248. Surgical Research Series, 1912–1913. Experiment No. 36. Surgical Pathology, No. 2394. Operation, February 11, 1913. Upon attempting to make a cavity in the usual manner the leg was fractured. A Lane plate was applied, with fair approximation of the two ends; and wound was sutured and the leg put up in plaster of Paris.

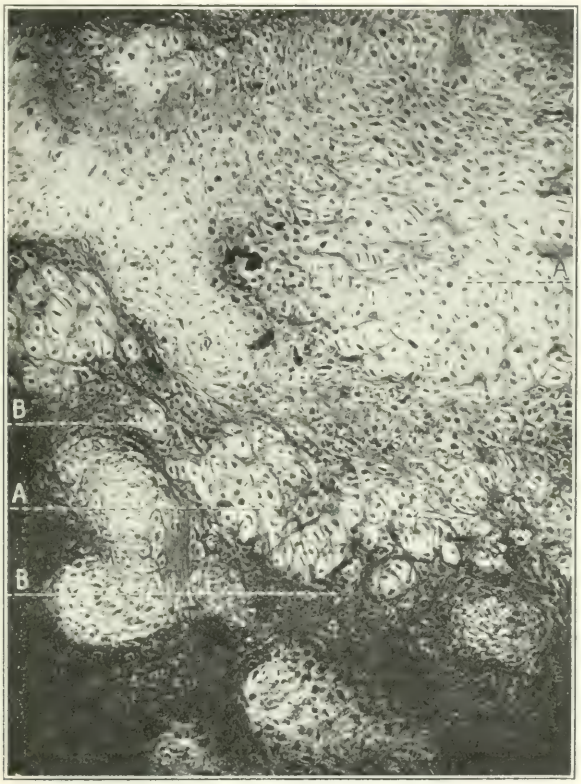

FIG. 15.—Surgical pathology, No. 2394. Repair of fracture, under high power. *A*, cartilage; *B*, bone.

March 4 the cast was removed; wound healed. There was, however, no union, so the dog was killed with chloroform. Duration since fracture, twenty-two days.

Autopsy. The ends of the bone are thickened and club-shaped, and have considerable callus around them. Longitudinal sections made through the ends of the bone.

Microscopic Examination (Fig. 15). Here under high power one sees again gradations between connective tissue, cartilage, and bone.

CONCLUSIONS. In this series of experiments two types of repair of the bone have been observed:

1. Where the periosteum has been well stripped off the bone for a distance of several centimeters it is separated from it by serosanguinous fluid. Here the bone has apparently been repaired from the medullary cavity by a framework of connective tissue, upon which calcium salts have been deposited, forming osteoid tissue; there has also been a reparative process from the connective tissue of the Haversian canals at the sides of the bone incision.

2. Where the bone has repaired under a definite fibrous connective-tissue capsule, which is continuous with periosteum and also adherent to the surrounding muscles. This connective tissue is apparently not different from other connective tissue, except that in immediate approximation to the newly forming bone there is a single layer of cells which are larger in shape and have nuclei that take a heavy stain. In places there is a metaplasia from the connective-tissue cells to these osteogenetic cells.

In the process of repair in fractures by the output of callus there is a metaplasia from connective tissue which seems to arise from the surrounding tissues rather than being a direct continuation of the periosteum. There is also a direct metaplasia from a tissue closely resembling fibrocartilage into young bone.

In attempting to draw clinical deductions from these experiments in regard to transplantation of bone, it is reasonable to assume that the periosteum acts as a limiting membrane to the bone, tends to conserve its shape, and to furnish its blood-supply. If the periosteum is stripped from the bone of the transplanted part, numerous thrombi will occur, where the vessels enter the diaphysis; but if the periosteum is left attached, the blood-supply will probably be more quickly established through the vessels of the periosteum.

If small pieces of bone are transplanted, it is obvious that Haversian canals must be cut in many directions, thus allowing easy ingress to the newly forming bloodvessels. The fragments also must receive a good blood-supply from the entire periphery. They grow in this case without the aid of periosteum.

I wish to extend my thanks to Drs. Clarke, Auchincloss, and Prime for many kind suggestions and valuable advice, and to Dr. McWhorter and Mr. Agnew for making the photomicrographs.

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A COURSE IN SURGICAL TECHNIC*

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The course in surgical technic here described was designed to meet a demand in the surgical training given in Columbia University. It is realized that familiarity, by actual observations on animals, with the use of aseptic materials, the appearance and manipulation of living tissues, the control of hemorrhage, and the methods employed to prevent contamination and subsequent infection of recent wounds, is an equal advantage to the graduate whether he enters practice immediately on graduation or prepares himself further by a hospital training. The opportunity to participate in operating-room routine, such as the preparation of the patient, the surgeon, the instruments and materials, is now of recognized value, and this recognition has created a need for special training in the details of aseptic surgical technic.

The course is given to fourth-year medical students in the Laboratory of Surgical Research, where there is a well-equipped animal hospital. In order to provide an opportunity to educate recent hospital graduates in teaching methods as well as to instruct the students, the teaching staff has been made unusually large. It includes twelve instructors in surgical technic and two in surgical pathology, all of whom have had surgical service in a hospital.

Four of these instructors are on duty at the same time. Each instructor serves for three months, but, by a scheme of rotation, he acts as assistant instructor during the first two, supervising the students at one

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table where he fills the place of second assistant, while during the third month he is instructor in chief and attending surgeon at the University Animal Hospital, where he makes a daily visit. The schedule is so arranged that each instructor becomes the chief twice in a period of twenty-four scholastic months.

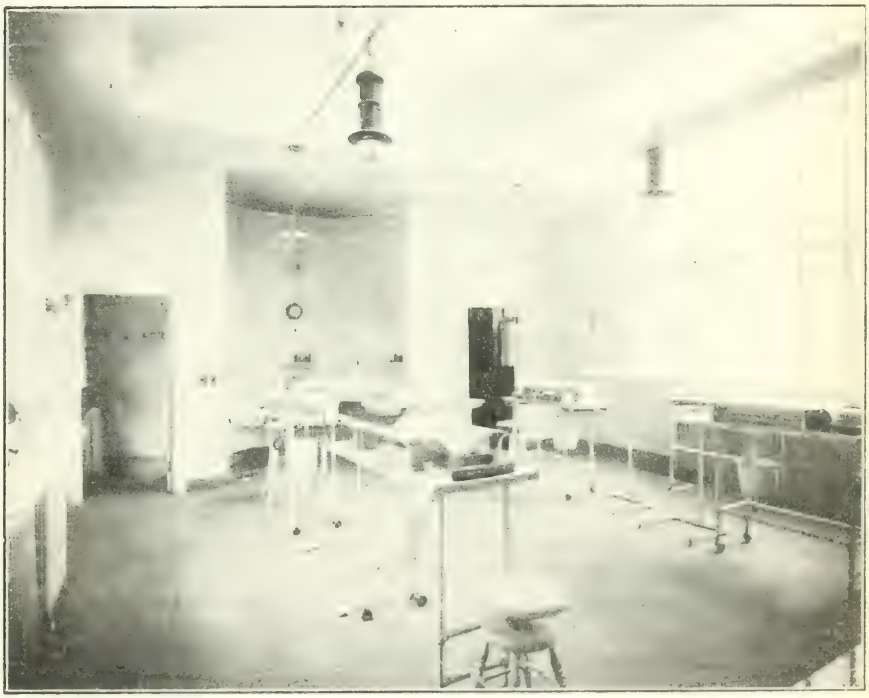
The course is an optional one, but so great is the interest it has aroused that at least three-fourths of the fourth-year students elect it, and each of the sections into which the class is divided contains ten men on an average. Each section receives instruction for eight periods of two hours each. It is divided into groups of three men, so that an average section furnishes operating staffs for three tables, and the men at each table rotate at successive lessons in the position of anesthetist, first assistant and operator.

The nurse in charge is a graduate of a hospital training-school. She is familiar with all surgical requirements and has supervision over the animal hospital and operating-room, under the direction of the attending surgeon on duty. At its initial lesson she instructs each section in the preparation of surgical supplies and their sterilization, the care of instruments and utensils, and in the manipulation of sterile gowns, gloves, etc. She also prepares for the operative sessions exactly as in the operating pavilion of any first-class hospital.

The operating-room is furnished and equipped on modern lines. It is 20 feet square, lighted by side-lights and skylights, and provided with powerful arc-lights. Adjacent rooms are used for sterilizing, anesthesia and private operations, and there is a detached room for necropsies. The sterilizing plant includes an autoclave, instrument boiler, utensil boiler and water sterilizers of the latest type.

The animal hospital has been fitted up with every regard for the animals' comfort. Each animal has a separate cage, and there are especially heated ones for recovery from anesthesia. The entire time and attention of one man is given to these animal patients. The animals themselves, as well as their surroundings, are kept scrupulously clean with the aid of a bath-tub, sluicing-hose, proper disinfectants and abundant light and ventilation. As the hospital is maintained to treat animals with surgical lesions, many dogs suffering

from tumors, infections and prolapsus uteri have been received as private patients. Thus the students under direction of the attending surgeon have had the opportunity of assisting at operations on real pathologic conditions. In his daily visits the attending surgeon inspects all the animals and gives such directions for their care as may be necessary to the nurse in charge



Operating-room for the class in surgical technic, Department of Surgery, Columbia University.

and her assistant. Daily notes of temperature and general condition are entered on each patient's chart for the information of the surgeon and the students.

In order to preserve uniformity of instruction, the professor of surgery has prescribed a definite series of demonstrations and operations for the course. A printed outline of these is furnished to every student at his first lesson.

First Session: The course opens with a lecture and demonstration on (*a*) suture materials and instruments; (*b*) preparation and sterilization of surgical supplies; (*c*) the administration of anesthetics to animals; (*d*) the preparation of the animal for operation; (*e*) the sterilization of the surgeon's hands, and relative values of different methods in use, and (*f*) prescribed method of putting on cap, mask, gown and gloves.

Second Session: The abdominal incision and its repair in layers. Repair of wounds in the intestine. Enterotomy performed, and intestinal suture illustrated. Technic of applying dressings.

Third Session: Lateral entero-enterostomy.

Fourth Session: Posterior gastro-enterostomy.

Fifth Session: End-to-end entero-enterostomy, by suture.

Sixth Session: Exploratory laparotomy, for a stab-wound of the abdomen, inflicted under anesthesia. The location and repair of the lesions are demonstrated.

Seventh Session: Nephrectomy, illustrating the treatment of the pedicle.

Eighth Session: Repetition by each table of any operation which has been unsuccessful.

The routine of a session is as follows: The chief instructor outlines briefly, at the beginning of the lesson, the steps of the operation to be performed, illustrating them by diagrams on the blackboard. He then assigns the students to their duties, and the usual routine of an operating-room begins. The anesthetist starts the anesthesia in the etherizing-room, the animal is placed on the operating-table and the field prepared by the first assistant (the hair has been removed and the animal given a full bath on the day before by the attendant). The skin is prepared by scrubbing with green soap and water, then with alcohol 70 per cent., and rinsing with mercuric chlorid 1 : 10,000. The table is then wheeled into the operating-room and draped with sterile sheets by the operator, who has previously scrubbed up and has put on cap, mask, gown and gloves, under the supervision of his instructor.

During the operation the instructor at each table directs every step and calls particular attention to the observation of asepsis, handling of instruments, placing of sutures, control of hemorrhage and the importance of handling living tissues with gentleness. Before leaving the room the student operator writes

a description of his procedure on the history sheet of the chart, which is to hang by the animal's cage.

At the conclusion of the operation, every step of which has been done under full anesthesia in every case, suitable dressings are applied to the wound and the animal is carried to the hospital and placed in a "warming cage," in order that chill may be prevented and the animal made comfortable. The animal receives special attention during convalescence, its temperature and general condition are noted daily on the chart, and the diet is carefully regulated by the attending surgeon. The student-operator is urged to visit his convalescent patient and to consider the outcome of his procedure of importance equal to that of the operative result on a human being.

At the beginning of each operative session, necropsies are performed by one of the instructors in surgical pathology on any animals which have died as a result of operation. A few, selected from the convalescents, are chloroformed and necropsied for the purpose of showing the students the contrast between the normal repair at various intervals after operation, and the pathologic conditions produced when an operation has been unsuccessful. Attention is drawn to the resistance of the healing wound to tension, in both abdominal and intestinal walls, to the constant formation of peritoneal adhesions, and to the benefit or harm which may result from the attachment of the great omentum to the various viscera. Preceding a necropsy on a pathologic animal, the student who operated on it is questioned as to his procedure, and as to any postoperative symptoms which might indicate the cause of death. He is also questioned regarding the treatment he would pursue with a human patient who exhibited similar signs and symptoms, and is asked to give his reasons therefor. Thus a constant effort is made to keep clear in the minds of the students the clinical aspects of the case, and the importance of necropsies is emphasized, as a means of correlating them with the various pathologic conditions and processes that are found to exist. This practice is intended as an aid to the interpretation of physical signs and to the understanding of the principles of treatment in human surgery.

Specimens of tissues are selected at the necropsies, and microscopic sections are made to show the students the cellular morphology of all common post-operative conditions, including wounds healing by first intention, infected wounds, and the various types of peritonitis, with or without adhesions. At the close of each necropsy, the student-operator is required to write a complete report of the pathologic findings, and these reports are bound with the histories. To increase the interest of the students, a comparative record of the results of each section's work is kept, which proves a stimulus to both students and instructors.

The routine performance of necropsies has brought out many interesting surgical facts which should be of lasting benefit to the students. Peritonitis occurred most frequently after the operation of end-to-end intestinal anastomosis. In the cases of gastro-enterostomy the most frequent cause of death was pneumonia, suggesting the increased percentage of pneumonia due to surgical interference in the upper abdominal cavity. To indicate the value of pathologic material that may occur during the year and the benefits that may be derived from the study of such material, a few of the unusual causes of death occurring with the various operations are briefly mentioned here:

1. *Gastro-Enterostomy.*—One case of enormously distended stomach with contracted intestines, due to a small band with a valve-like action, causing obstruction to the entrance to the jejunum. One case of death due to inanition caused by anastomosis of the stomach to the descending colon; here sufficient care had not been taken to identify the loop of intestine used for the anastomosis.

2. *Enterorrhaphy.*—One case of gangrene of the intestine in which the intestinal clamps had been too tightly applied.

3. *End-to-End Entero-Enterostomy.*—One case of cirrhosis of the liver with ascites; death was due to oozing. One case of infection of the abdominal wound, followed by protrusion of the omentum with beginning gangrene.

4. *Exploratory Laparotomy for Stab-Wound.*—One case of general peritonitis, due to an overlooked stab-

wound in the intestine. One case of thrombosis of the superior mesenteric vein, with gangrene of the small intestine. This was probably due to excessive traction on the mesentery, as no other cause could be found. One case of general peritonitis due to a gauze sponge that had been left in the abdominal cavity.

The sections that saw these last three necropsies learned a lesson in surgery which they will probably never forget, so clearly was brought out the necessity for careful handling of the tissues, and for keen systematic observation.

Intussusception was found to be a frequent cause of death, and has apparently no relation to the type of operation performed. It is probably due to the handling of the intestine. Both cross-sections and longitudinal sections have been made to show the students the relations of the layers of intestine and constriction of the mesentery in such conditions.

The private animal patients admitted to the hospital have been the means of demonstrating several neoplasms as well as other instructive conditions. One student section had the opportunity of operating on congenital oblique inguinal hernia occurring in three youthful pigs, all from the same litter. The students had the subsequent satisfaction of having these three animals returned home, discharged cured, and the wounds healed by first intention.

The advantages of a course of this kind are numerous. The existence of a staff of instructors, each occupying in turn the position of chief, not only makes each instructor's service short enough to maintain constant enthusiasm, but also acts as a stimulus, because of the element of competition. Each surgeon has an opportunity to observe the teaching methods of his associates, and to note their merits or defects, with a view to increasing his own efficiency. The combination in a single course of the technic of operative procedure with the pathologic findings in the cases in which operation has been performed leads the student, when brought into contact with clinical cases, to reason in terms of pathologic processes and conditions. This assists him to correlate his knowledge of anatomy, pathology, bacteriology and physiology with surgery. He is trained to base his conclusions on observations of facts rather than on dogmas.

Finally, after such a course the student has become acquainted with the technic of asepsis, the use of surgical instruments and the other essentials of general surgical procedure; he has learned that an operation must be conducted exactly and methodically, and he has felt a personal responsibility for its success or failure.

When, therefore, he enters practice and is confronted with the necessity of operating, he is no longer an untried apprentice. Already familiar with the fundamental principles of aseptic surgical work, he is enabled to avoid many a costly mistake, and is far better fitted to do credit to himself and justice to his human patients.

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RELIABILITY OF DOGS AS SUBJECTS FOR EXPERIMENTAL NEPHRITIS.*

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That "spontaneous" chronic nephritis is common in dogs is generally acknowledged, but its frequency is perhaps underestimated. The following observations were made during the preliminary stages of work on experimental nephritis. Dogs were utilized which had been or were subsequently used as subjects for nephrotomy in the student course of surgical technic. The pathological findings have been confirmed by the routine examinations of the laboratory. In each case a section was removed from the upper pole of one kidney and studied microscopically, while the urine was examined daily in as many cases as possible. The dogs were kept in metabolism cages, with measured water and in many cases weighed food supply, so as to avoid, as far as possible, fluctuations, in specific gravity and otherwise, due to controllable factors. The renal specimens removed by the students were in a few instances too small to be entirely satisfactory for examination and to give a definite idea of the extent of the lesions found. The tissues were fixed in formaldehyde (5%), embedded in celloidin, and stained with Delafield's hematoxylin and eosin. In some cases Mallory's aniline blue stain for connective tissue was also used to confirm the degree of fibrosis.

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These kidneys may be divided into four groups according to the degree and extent of histological change observed. Briefly stated these lesions were as follows:

1. Chronic Nephritis.

Nos. 2185, 2234, 2235, round-cell infiltration, increase of connective tissue.

No. 2186, round-cell infiltration, increase of connective tissue, atrophic glomeruli.

No. 2388, round-cell infiltration, increase of connective tissue, glomerular nephritis.

No. 2431, glomerular nephritis.

No. 2470, slight increase of connective tissue, glomerular nephritis.

No. 2325, same as 2470, with casts in collecting tubules and descending limbs of loops of Henle.

No. 2471, same as 2470, with sclerosis of arteries.

No. 2326, slight round-cell infiltration, glomerular nephritis.

2. Slight Chronic Nephritis.

Nos. 2046, 2429, 2475, slight glomerular nephritis.

No. 2385, same, with casts in collecting tubules.

No. 2236, slight round-cell infiltration, slight glomerular nephritis.

No. 2327, slight increase of connective tissue, glomerular nephritis.

No. 2233, slight round-cell infiltration, slight increase of connective tissue, atrophic glomeruli.

3. Nearly Normal.

No. 2430, slight increase of connective tissue, capsule slightly thickened.

No. 2428, one atrophic glomerulus (constant albuminuria; autopsy showed round-cell infiltration and increase of connective tissue).

No. 2474, slight round-cell infiltration, hyaline casts in tubules, few glomeruli show connective tissue thickening of capsules.

4. Apparently Normal.

No. 2328, cortex only, in specimen; died after operation, no autopsy.

To summarize:

Chronic nephritis,	10 cases	or 47.6 per cent.
Slight chronic nephritis,	7 "	" 33.3 " "
Nearly normal,	3 "	" 14.3 " "
Apparently normal,	1 "	" 4.8 " "

Four other dogs were discarded without operation because the urine showed marked evidences of renal disease: 269A,

low specific gravity, albumin with casts; 270A, high specific gravity, casts; 271A, 272A, high specific gravity, albumin, casts. If these be included, a series of twenty-five dogs shows only four per cent. of apparently normal kidneys.

Of the twenty-one dogs whose kidneys were examined, seventeen showed distinct or slight lesions of chronic nephritis, increase of connective tissue, with or without round-cell infiltration, glomerular atrophy or increase of glomerular nuclei or capsular thickening. In two of the three dogs classed as nearly normal histologically the urinary findings were inconclusive in one, because of the occurrence of mange with local application of drugs; the other showed low specific gravity and a trace of albumin, so the kidneys of these dogs could not be considered as normal. One dog remains, and in this case, apparently normal histologically, the renal section consisted of cortex only.

To be of diagnostic value, sections must (1) include medulla as well as cortex, for lesions are often found in the medulla or near the apices of the cortical pyramids; and (2) they must be of fair size, since lesions may not be generalized and a small section may fail to show signs of disease.

Urine examinations were made in twelve of the twenty cases which showed histological renal lesions and two of these (Nos. 2429, 2233) must be excluded because of complicating distemper or mange with local application of a renal irritant drug. Of the ten remaining, eight (Nos. 2185, 2234, 2186, 2326, 2046, 2475, 2385, 2428) showed low specific gravity and seven of these (excepting No. 2046) a trace of albumin also, with casts in two instances (Nos. 2186, 2385). In one (No. 2470) there were abnormally high specific gravity, large amount of albumin, many fatty and hyaline casts and persistent marked lipuria. These urinary findings though constant cannot, however, be claimed to be indicative of "spontaneous" renal disease, because in six (Nos. 2185, 2234, 2186, 2326, 2046, 2475) resection of one kidney had been performed before urine examinations were begun and the effect of this renal traumatism cannot be disregarded.

We may, however, say that there is no evident disagreement between the conclusions drawn from examinations of a section of renal cortex and medulla and those from repeated careful urinary examinations.

Of the twenty-five dogs examined only one (or 4%) had apparently normal kidneys. From a strictly histological standpoint, a normal kidney was found in only one of the twenty-one dogs (4.76%), and the specimen in this case was confined to the cortex.

This immense preponderance of abnormal kidneys in even so small a series of dogs as twenty-five would lead to the conclusion that chronic interstitial and glomerular renal lesions are so common in the class of dogs available for laboratory use that little confidence can be placed in experimental results whose significance depends upon the assumption that the experiment was performed upon an animal with normal kidneys. Since such lesions seem to be almost of universal occurrence in dogs one must take this into consideration in interpreting lesions which are the apparent results of experimental renal irritation, and must not draw too positive conclusions that such lesions are actually the result of the experimental work.

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ON THE LYMPHATIC DRAINAGE OF THE PERITONEAL SAC

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The lymphatic drainage lines from the subdiaphragmatic viscera are factors of which we have some definite knowledge. The subperitoneal, submucous and interlobular visceral lymph capillaries drain through collecting radicles into definite groups of neighboring lymph-nodes and from these nodes efferent vessels eventually empty into the lumbar trunks, receptaculum or thoracic duct.

Clinically, the anatomical relation that definite groups of nodes bear to definite organs and areas and to each other enters into the pathological conception of all infections of the abdominal cavity and visceral malignancy. Metastatic deposits in tissue distant from a primary carcinomatous focus are observations too common to arouse comment. Malignant invasion of the liver from the breast by means of a retrograde permeation of the epigastric or costoxiphoid lymphatic channels has long prompted surgeons to adopt the routine removal of these avenues of extension in operations for carcinoma of the breast. Deaver and Pfeiffer (*A. J. M. Science,* 1912) consider clinically the important relation between infections of the gall-bladder and pancreatitis and the lymphatic intercommunication of gall-bladder with duodenum and pancreas. Infections of the appendix may travel far indeed when the spread of its outgoing lymph stream may be known to reach the upper abdomen.

Sweet and Stewart (*Surg., Gynec., and Obst.,* vol. xviii, 4) offer considerable evidence that the ureteral submucous and subperitoneal lymph-channels are the avenues of extension for infectious processes from the bladder to the kidney and not the ureteral lumen.

On the other hand, the peritoneal sac, so far as we know, having but a limited lymphatic function to perform under normal circumstances, seems to possess distinct and extremely active potential lymphatic absorption lines. These appear to be independent of those of the visceral system and not confined to the central tendon of the diaphragm, as Muscatello taught in 1895, but are present over the whole surface of the dome of the diaphragm.

Poirier and Cuneo have described the diaphragmatic lymphatics as forming glandular groups on the convex or pleural surface of the diaphragm, arranged as a few small nodes posteriorly between the crura, two small middle groups placed laterally and several anterior groups including the costoxiphoid glands of Sappey. These latter anterior groups receive afferents from the liver through the falciform ligament and send efferents to the retrosternal chain of lymphoid tissue. The hepatic radicles from the falciform ligament of the liver unite in a short trunk at the ensiform to penetrate the diaphragm and empty into the costoxiphoid nodes.

From the internal mammary chain efferent lymphatics join the thoracic duct at or near the subclavian vein or enter the subclavian independently. Efferent channels from the posterior and lateral diaphragmatic groups are said to drain posteriorly into the juxta-aortic nodes or direct into the duct. Afferent vessels to these diaphragmatic nodes are not mentioned. The problem of investigating lymphatic drainage from the peritoneal sac has been studied by Von Recklinghausen, Poirier and Cuneo, MacCallum, Muscatello and many others and has been carried on by means of the injection of foreign agents into the peritoneum and histologic study of the diaphragm.

Prominent among the questions that arise in a consideration of this subject are:

1. The physical integrity of the mesothelium covering the abdominal aspect of the diaphragm, as to the presence or absence of openings of sufficient size to be called stomata (Von Recklinghausen, 1865).

2. The exact manner and path of absorption from the peritoneal cavity of injected foreign agents.

3. The existence of a direct lymphatic absorption as opposed to or in conjunction with a hæmatogenous absorption.

4. The activity of the diaphragmatic lymphatics in assuming the major rôle in such absorption.

Question 1: No observer since Von Recklinghausen has been able to offer any satisfactory evidence of openings in the diaphragmatic peritoneum, by virtue of which the peritoneal sac becomes a unit with the lymphatic system. MacCallum (*Johns Hopkins B.*, vol. 14, 146) reports a most complete histologic study of the diaphragmatic peritoneum and finds no evidence of such stomata. After repeated and careful study of the flat-celled mosaic which covers the diaphragm the writer wishes to confirm this report. Tangential, protargol impregnated sections (Fig. 1) show no break in the perfect approximation of cell to cell, of sufficient size to be in any sense considered as a mouth or stoma.

Furthermore, it seems to the writer of little pathological consequence whether it is generally believed that such openings do exist or whether, as occurs in the metabolism of the vegetable cell, certain foreign substances are abundantly able to migrate intra- and intercellularly by means of a process of cell inclusion and extrusion described by Pfeiffer (*Physiology of Plants*, vol. i, p. 3) as the translocation of foreign agents by streaming movements of the cell protoplasm.

Question 2: Muscatello (Virchow's *Archives*), 1895, and Buxton and Torrey (*J. Med. Research*, vol. xv, No. 1, July, 1906) made the remarkable observations that finely divided particles of carmine, carbon, chicken blood corpuscles and dead micro-organisms were transported from the peritoneal cavity of animals to the superior retrosternal lymph-nodes at the junction of the first rib and the sternum in five minutes. The writer has conducted similar experiments fifty or more times and has found that not only does India ink and fine lampblack reach these distant lymph-nodes (Fig. 2) from the pelvic fossa with this remarkable speed, but in some instances reaches the bronchial lymph-nodes as well. There seems to be no doubt, therefore, that there exists a prompt lymphatic absorption from the peritoneal sac by certain lymph-vessels which carry the absorbed material as far as the superior retrosternal lymph-nodes.

The path followed by this absorbed material in migrating from the pelvic fossa to the superior retrosternal lymph-nodes is more difficult to demonstrate. MacCallum's study (*vide supra*) was concentrated upon the double-layered cellular membrane constituting the diaphragmatic mesothelium and the lymphatic wall, through which the absorbed agents must pass in order to leave the peritoneal sac and enter the lymph stream. His conclusions were, that phagocytosis played the primary part in this transmigration and that a certain small percentage of the material might pass free through a loosely constructed intercellular substance into the lymphatic lacunæ. With these conclusions of MacCallum in mind, namely, the predominance of a phagocytic agency in the emigration of such agents as carbon through the peritoneum covering the diaphragm, and those of Muscatello and Buxton and Torrey, relative to the remarkably rapid dissemination of such foreign agents to the retrosternal lymph-nodes, the writer proceeded to study not only the peritoneal mesothelium but the paths taken by the carbon after it had penetrated the peritoneum, at all possible points from the peritoneum to the retrosternal glands. From 3 to 5 c.c. of a suspension of artist's special moist lampblack in salt solution were injected into the lowest point possible of the peritoneal sac of guinea-pigs. After vary-

ing periods of time the animals were killed and the diaphragm, superior retrosternal lymph-nodes, mesenteric nodes, and portions of the pelvic and abdominal parietal peritoneum were removed.

The microscopic investigations included the study of tangential sections of the abdominal surface of the diaphragm at various points of the muscular edge and tendinous centre, similar sections of the pleural surface of the diaphragm, and cross-sections from each of several areas. From these sections, photomicrographs are selected and presented herewith to illustrate the conditions found. Examination of the unsectioned peritoneal surface of the diaphragm with binoculars shows the particles of carbon to be gathered in smudges over its entire surface and attempts to either wash it off or otherwise remove it demonstrate its firm adhesion to the glistening peritoneal surface. Tangential celloidin sections of this abdominal peritoneum show, as MacCallum says, a number of carbon particles within leucocytes, but in periods of less than ten minutes after injection, the greatest quantity of carbon by far is to be seen free within the peritoneal cells and in the lacunæ. At points other than where the lucunæ appear (Fig. 3), the carbon, having penetrated the peritoneal mesothelium, is seen in the endomysium among the muscle fibres. In these endomysial trabeculæ the lymphatics of the diaphragm are known to run and in section they are seen to divide and join again, forming a network of carbon-containing tissue tracts over the whole diaphragmatic musculature.

In cross-section at any point in the diaphragm the carbon is seen to (Fig. 4) penetrate the peritoneal mesothelium and follow the endomysial channels from one side of the diaphragm to the other. Tangential sections of the pleural surface of the diaphragm present here and there (Fig. 5) large lymphatic radicals running just under the pleural mesothelium filled with carbon particles.

With the assistance of Prof. Adam Miller of the Department of Anatomy, the writer next attempted to inject the lymphatic radicles of the diaphragm with India ink by means of a fine glass cannula used by him in his vascular injections of the chick embryo. The ink when deposited under the mesothelium of the abdominal side of the diaphragm always followed fine parallel lines corresponding to the long axes of the tendinous and muscular fibres. When these lines are compared with the tangential sections (Fig. 3) they are seen to be the endomysial tracts which have been shown microscopically as the paths taken by the absorbed carbon.

After these injections under the peritoneal surface of the diaphragm a few distinct beaded lymphatic radicles appear on the pleural surface,

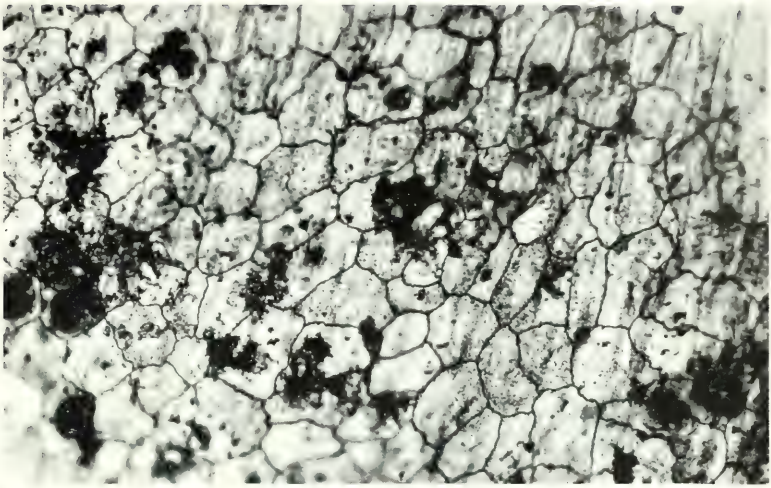

FIG. 1.—Tangential protargol impregnated section of diaphragm after injection of 3 c.c. of carbon. No stomata; carbon in smudges. (Photo. 450 diams.)

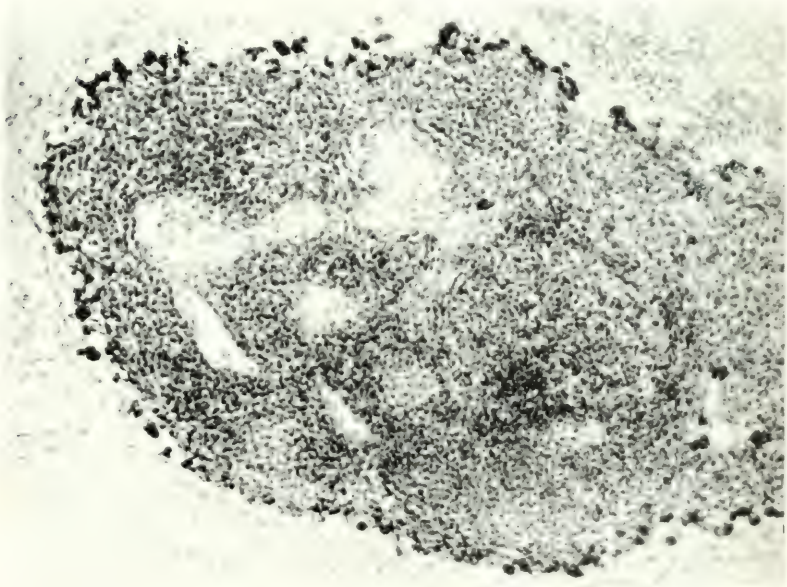

FIG. 2.—Superior retrosternal lymph-node, guinea-pig, five minutes after injection of 3 c.c. of carbon in pelvic fossa. Carbon in afferent sinuses. (Photo. 375 diams.)

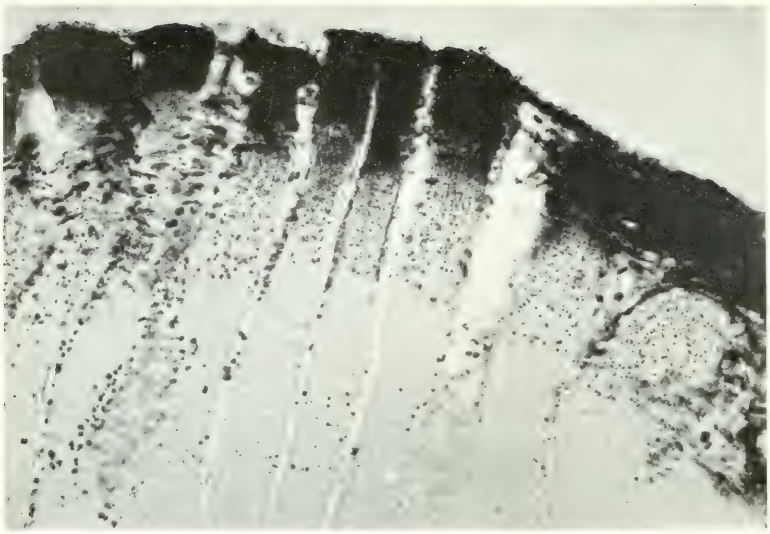

FIG. 3.—Tangential prolonged impregnated section of peritoneal surface of diaphragm, showing carbon in endomysial channels.

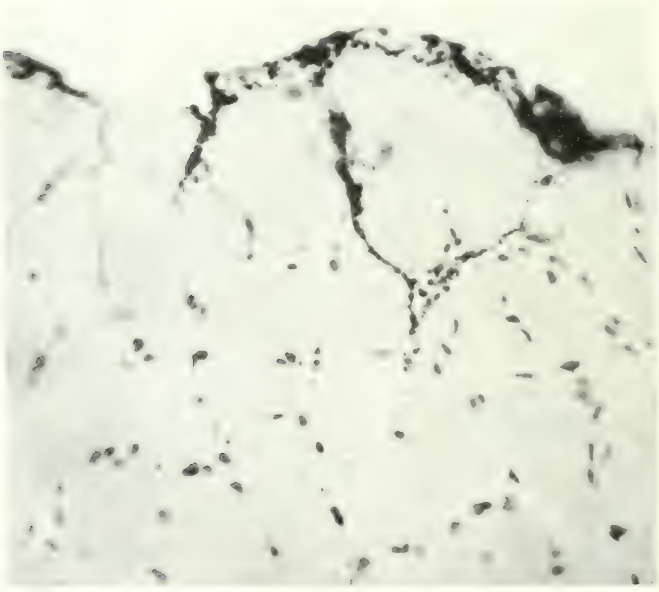

FIG. 4.—Cross-section of diaphragm, showing carbon five minutes after injection penetrating well into endothelial channels from peritoneal surface. (Photo., 400 diams.)

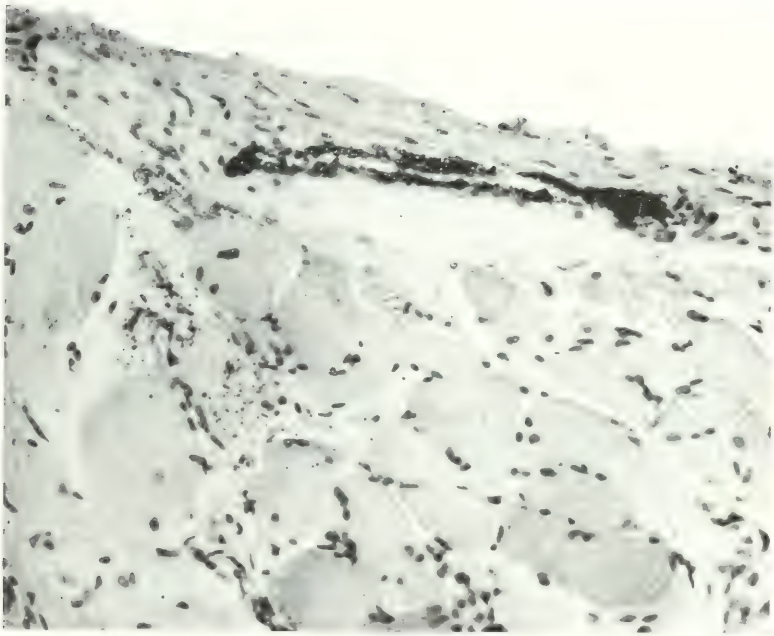

FIG. 5. Cross section, pleural surface of diaphragm, showing lymphatic filled with carbon five minutes after injection and carbon collecting in endothelial channels near pleural surface. (Photo. 300 diams.)

coursing from the muscular and tendinous portions to the ensiform attachment. Similar radicles rapidly carried the ink from the region of the liver up through the falciform and to the costoxiphoid glands of Sappey. Attempted retrograde injection of the retrosternal lymphatic chain from the superior retrosternal glands to the ensiform and diaphragm failed apparently on account of valves in the lymph-vessels.

Question 3: The observations made by Muscatello and Buxton and Torrey already referred to, verified and amplified by the writer, made practically positive the belief that a direct lymphatic absorption does take place from the peritoneal sac. Recently the question of hæmatogenous *versus* lymphatic absorption from the peritoneal sac has been brought forward in a report by Dandy and Rountree, ANNALS OF SURGERY, April, 1914, in which a general denial of any considerable lymphatic absorption was made. Without occupying space by going into details as to possible physico-chemical errors consequent upon drawing conclusions from a comparative test of the time of appearance of sulphone-phthalein in the urine and in the thoracic duct, the following points may be noted:

Wells and Johnstone (*J. of Infec. Diseases,* 1907, vol. iv) have successfully attempted to show that bacteria do not pass into the blood-vessels of the general peritoneum but reach the blood wholly by way of the lymphatic vessels. In order to approach the conditions active where fluids are to be absorbed from the peritoneal sac, the writer injected with the same technic used for carbon, 3 c.c. of a 0.1 per cent. solution of trypan blue. The animal was killed immediately and in the short space of time necessary to remove the superior retrosternal lymph-nodes, namely, two minutes, these were found a deep blue color.

Time had not elapsed for any general diffusion of the dye and we feel justified in concluding that it had travelled from the pelvic fossa by the same channels as the absorbed carbon.

Question 4: In all the carbon and trypan blue experiments various specimens of tissue that had been directly bathed in the injected solutions, namely, the parietal peritoneum other than that of the diaphragm, the visceral peritoneum and the mesenteric glands were investigated microscopically for evidence of absorption at these points and none was found. From the peritoneal sac, therefore, solid particles of carbon, living and dead microörganisms, chicken blood-corpuscles and saline solutions of trypan blue are seen to be absorbed definitely by lymphatics which, having their origin in the diaphragm, stream past the superior retrosternal lymph-nodes and in such short space of time as

to prove this absorptive path one of definite and prompt activity whether a coincident hæmatogenous absorption takes place or not.

Carbon injected under the visceral peritoneum regularly followed definite lines to definite neighboring lymph-nodes and in one instance, where the cæcum was especially infiltrated, a gland taken from the region of the duodenal curve near the pancreatic head was found full of carbon.

Incidentally the rapidity with which fluids are removed from the peritoneal sac under normal conditions of circulation was noted in the first place by the remarkably small residue of 10 c.c. of carbon solution that could be found in the peritoneal sac twenty-five minutes after injection; and in the second place by observation made in the rabbit, when two drachms of the animal's blood was allowed to flow from a mesenteric vein into the pelvic fossa and, without further damage to the peritoneum, the abdomen closed. In twenty-four hours all that could be found of the two drachms of blood was a small speck of pinkish fibrin, which consisted microscopically of a few leucocytes and a few strands of hæmoglobin stained fibrin.

Conclusions.—1. Absorption of certain solid foreign material injected into the peritoneal sac occurs with marked rapidity, first by a process of translocation through the cells of the diaphragmatic mesothelium and later through the agency of leucocytes.

2. That such solid foreign material having passed the peritoneal mesothelium is conveyed through the endomysial tracts throughout the diaphragmatic musculature to the lymphatic radicles on the pleural surface of the diaphragm, from these through the various diaphragmatic gland groups to the costoxiphoid glands of Sappey, and from thence through the retrosternal chain of lymphoid tissue to the subclavian vein or thoracic duct.

3. That certain fluids injected into the peritoneal sac follow the same lymphatic absorption lines, whether they coincidently enter the blood stream directly or not.

4. That the tissues of the diaphragm take a distinctly active part in absorption from the peritoneal sac and that other areas of parietal peritoneum functionate little if any in the lymphatic absorptive process.

5. That the post-operative postural treatment of pelvic peritonitis as advocated by Fowler has definite pathological foundation.

The author's gratitude is here recorded, for the friendship that prompted the assistance given him by Prof. W. C. Clark, Adam Miller and Dr. John C. McWhorter.

THYROID AND CIRCULATORY CHANGES FOLLOWING EXPERIMENTAL LIGATION OF THE THYROID VESSELS.*

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Although many methods of experimentation have been employed in order to induce changes in thyroid function, no studies dealing with the effects of interference with the vascular supply of the thyroid gland were found in a careful examination of the literature. Experiments directed towards blocking the arterial and the venous supply of the gland theoretically offered a favorable opportunity to learn if, on the one hand, pathological changes similar to those found in the abnormal human thyroid, and, possibly, some of the clinical manifestations of "hyperthyroidism," would result, or, on the other hand, if such changes and manifestations bore no relationship to interferences with the thyroid circulation.

It may be stated at once that, in the earlier experiments, begun in the spring of 1913, very striking changes in the thyroid lobes were noted after ligation of their veins. However, additional experience showed that profound alterations could result from slightest errors in technic or asepsis, and many difficulties were encountered in the interpretation of the findings. Some variations in the size and shape of the acini and in the amount of interacinar tissue were found in the thyroids of presumably normal animals. Furthermore, it became evident that the pulse rate of normal dogs varies within considerable limits, and that dogs often have a tremor-like movement of the entire musculature simulating the tremor of "hyperthyroidism." For these reasons a number of apparently contradictory observations were made, and much of the work was necessarily discarded. These remarks may serve as explanation of the fact that the results I have

* Received for publication May 17, 1915.

obtained are not uniform, even at the present time. However, certain positive and negative findings should be recorded.

Technic. — All the experiments were performed upon dogs under full ether anesthesia. Animals of approximately the same size and age were chosen. Their nourishment before and after operation was fairly uniform in quality and quantity. Hair covering the front of the neck was removed with barium paste the day before operation. The field of operation was thoroughly cleansed with soap, alcohol, and 1 : 1000 bichloride solution after the animals were anesthetized.

The thyroid lobes must be exposed with the minimal amount of trauma and the lobes themselves should not be touched during the operation. Any handling of the thyroids, or even a slight infection of the wound, is sufficient to invalidate the results. The technic of ligation of the thyroid arteries and veins bears such an important relation to the results obtained that the procedure I have found satisfactory will be described in detail.

A vertical incision is made over the middle third of the trachea. The musculature is separated in the median line, exposing the pretracheal fascia. The edge of the muscle of one side is grasped with forceps at two distant points and retracted. The thin pretracheal fascia can then be followed to the lateral aspect of the trachea. Here the thyroid lobe is brought into view by pressing gently forward against the posterior aspect of the carotid sheath. The thyroid lobe shimmering through the lateral tracheal fascia will be recognized by its shape and pinkish color. With the lobe as a guide the thyroid vessels are exposed by incising the fascia immediately beyond its superior and inferior poles. The vessels are then traced to the points at which the branches unite to form the main superior and inferior thyroid artery and vein. It is at these points that ligation should be practised, for the artery is then readily distinguished from the vein, and very few (generally two) ligatures are required to

block the chief arterial or venous supply of the lobe. The vessels are always divided between ligatures. All oozing of blood is controlled and the wound is carefully sutured in layers with fine silk.

Results of ligation of the veins of both thyroid lobes. — An examination made as early as one week after operation reveals no evidence of hemorrhage into the lobes. At the end of two weeks the acini are generally found much larger than normal, the interlobular connective tissue is compressed between the acini, colloid stains faintly, the veins are distended.

Six weeks after operation the following picture was encountered: Most of the follicles are small, irregular, shrunken. There is some increase of, and scattered areas of hyaline degeneration in, the interacinous connective tissue. The acini appear increased in number, the additional follicles being of small size. The walls of the veins show connective tissue thickening. The picture appears to be a combination of interstitial thyroiditis and scattered areas of hyperplasia. This was not met regularly, however. On the contrary, the changes were often insignificant; variations from the normal were frequently so slight that constant comparisons with sections of normal thyroid glands were necessary in order to estimate these differences. They consisted in a relative diminution in the size of the follicles combined with a relative increase in number, faint colloid staining in irregular areas, and patches of increased interacinar connective tissue.

Examination of the thyroid lobes three to six months after ligation of the veins disclosed fairly uniform changes, although these, as a rule, were slight. The average size of the acini was greater than the normal, there was evidence of coalescence of follicles, their lining cells were usually flattened, their outlines frequently indistinct, and the interfollicular connective tissue scanty. The walls of the veins were considerably thickened, the thyroid lobes were smaller and firmer and weighed somewhat less than the normal.

Variations in pulse rate were often but not regularly noted after ligation of the veins of both lobes. They were determined by frequent examinations and by comparisons with pulse rates before operation. Acceleration to one hundred and seventy beats in the minute was recorded; slowing of the pulse rate was never observed. When an acceleration was present it appeared within a week of the operation, persisted as long as ten weeks, and then gradually disappeared. It was never noted as late as four months after operation. Symptoms or signs referable to "hyperthyroidism," other than acceleration of the pulse rate, were not observed.

Results of ligation of the veins of one thyroid lobe. — The changes closely resembled those recorded after ligation of the veins of both thyroid lobes; they were, however, more regularly encountered and were often more pronounced after unilateral ligation. One remarkable feature was the fact that the unoperated lobe almost invariably showed the changes found in the lobe whose veins were ligated, although these changes were generally present to a lesser degree. A satisfactory explanation of this observation cannot be offered.

As early as two weeks after operation one specimen presented, upon microscopical examination, considerable irregularity in the size of the follicles (many being shrunken), faint staining of colloid, and a diffuse increase of interacinous connective tissue; these changes were found in both thyroid lobes, but to a greater extent upon the operated side. This picture was unusual, for the changes at the end of two weeks generally consisted in dilatation and irregularity of the acini and compression of the interacinous connective tissue; the differences in appearance between the operated and unoperated lobes being insignificant.

At examination made five to eight weeks after operation, many irregular, shrunken, and indefinitely outlined follicles were as a rule encountered; there was some increase of the interstitial connective tissue, which also showed areas of hyaline degeneration. The walls of the veins in and about

the operated lobe were thickened. These changes were generally present to a lesser degree in the unoperated lobe. The latter was usually somewhat larger and weighed a few milligrams more than its fellow.

The observations upon changes in the pulse-rate were analogous to those made after ligation of the veins of both thyroid lobes. The acceleration was not so great, however, the highest rate being one hundred and sixty-five to the minute.

Results of ligation of the arteries of one and of both thyroid lobes. — Remarkably few microscopical and gross alterations resulted. Whether the examination was made as early as two weeks or as late as three months after operation, the changes were invariably slight. They consisted chiefly in the production of a small rounded type of acinus, in both lobes when the arteries to both were ligated, and to a more marked degree on the operated side when unilateral ligation was performed. The alteration of the shape and size of the follicles manifested itself within twelve days of the operation, and was most evident in specimens examined six weeks after bilateral arterial ligation. Some sections showed moderate increase of the intralobular connective tissue. Slight diminution in size and weight of the thyroid lobe followed ligation of its arteries.

There was generally a retardation of the pulse-rate, whether one or both sides had been operated upon. This was never pronounced, the most marked slowing being eighty-nine to the minute. Changes in pulse-rate were more transient than those noted after ligation of the thyroid veins, for they generally disappeared within one month.

SUMMARY. — Careful technic and asepsis are essential in experiments conducted to determine changes resulting from interference with the blood supply of the thyroid gland. Uniform changes do not follow ligation of the thyroid arteries or the veins. When present, they are generally not profound. Alterations in structure are more common and more

marked after ligation of the thyroid veins than after ligation of the arteries. Following the former there is a decrease in the size of the acini; irregularity in their shape, increase of the interstitial connective tissue, and diminution in the amount of colloid. The end result, however (after three to six months), is an increase in size and coalescence of acini, scanty interfollicular connective tissue, and diminished size and weight of the gland. A reduction in size and generalized rounding in shape of the acini are the chief results of arterial ligation. The changes in one thyroid lobe, following ligation of its veins or arteries, may exist, to a lesser degree, in the unoperated lobe.

The only symptom or sign referable to "hyperthyroidism," following interference with the circulation of the thyroid lobes, consists in an acceleration of the pulse, of several months' duration, after ligation of the thyroid veins, and retardation of the pulse, of several weeks' duration, after ligation of the arteries.

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EXPERIMENTAL SUBARACHNOID INJECTIONS OF TRYPAN BLUE

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In considering the recently adopted subarachnoid administration of salvarsan as a therapeutic measure in the intensive treatment of luetic lesions of the central nervous system the following question among many others arises.

To what extent does salvarsan injected into the subarachnoid space reach the central nerve tissue with greater certainty and intensity than when administered intravascularly or subcutaneously?

The successful work of Professor Edwin Goldmann (Vitalfärbung am Zentralnervensystem) with trypan blue as a vital stain for the central nerve tissues suggested a way to answer our question.

The benzidine dye trypan blue differs from other vital stains such as isamin blue, in having the same chemotactic affinity for tissue as salvarsan and has therefore been selected as an indicator of the tissues with which the salvarsan injected subarachnoidally may unite.

After lumbar subarachnoid injections of various doses of a saline solution of the dye macroscopic and microscopic examinations of the central nerve tissues were made.

As a preliminary report covering observations made on twenty-six successful experiments with cats, dogs and rabbits the following findings are offered.

TECHNIQUE

Repeated subcutaneous injections of from 3 to 5 c.c of 2 per cent. saline solution of trypan blue.

Single intraarterial and intravenous injections of 10 to 20 c.c of trypan blue solution.

Single lumbar subarachnoid injections of varying doses of try-

pan blue from ½ c.c. of 0.1 per cent. solution in rabbits to 10 c.c. of 2 per cent. solution in large dogs.

The uniformly fatal and serious results reported by Goldmann following lumbar subarachnoid injections of trypan blue were not observed by the writer.

The few serious or fatal effects of intraspinal injections that did occur seemed to depend not upon the toxicity of the dye as used but upon the volume of the injection mass, the rapidity of its administration and the amount of spinal fluid that could be withdrawn prior to injection: In other words upon any factor of the technic which materially raised intraspinal pressure.

No anesthetic was administered, which in Goldmann's animals must have masked the reported central nerve symptoms, and when the dose of the injection mass was kept below a definite lethal quantity, 1 c.c. of a 0.1 per cent, solution in rabbits, no convulsions occurred or if they did appear in some animals, the immediate withdrawal of a small part of the dose allowed recovery in a few hours.

The difficulty of entering the lumbar sac with a fine needle in rabbits and cats rendered many experiments unsuccessful and necessitated special study of the anatomical conformation of their vertebræ. No laminectomies were necessary and only those animals are considered where subsequent autopsy showed a proper placement of the injection mass.

The spinal fluid of the rabbit and cat is so scant that only occasionally could a drop or so be obtained to identify the location of the needle point.

When the dosage of the injection was raised forcibly beyond that point which seemed to be sublethal for the animal, suddenly with very apparent release of intraspinal pressure, the animal became convulsive and simultaneously a deep blue color appeared in the eyes. This coloring of the eyes was a reflected blue not one of general conjunctival diffusion but of retinal infiltration as if the dye had burst its way from the spinal subarachnoid space into the cisterna subarachnoideæ and from there into the optic nerve sheath.

Repeated subcutaneous injections of varying doses of trypan blue continued over a period of several days and resulting in an intense intravitam stain for all other tissues of the body, failed to reach the central nervous system except in exceedingly small

quantities observeable macroscopically as a very faint sky blue in the cranial dura and the ventricular veins.

Intraarterial injections of sublethal doses of a 2 per cent. trypan blue solution were made into the carotid artery, the animal allowed to live for forty-eight hours and then killed. At autopsy the generalized blue color was marked. In contrast was the notable creamy whiteness of the unstained central nerve tissues with only slight tinting of the cranial dura and ventricular plexuses.

Intravenous injections of sublethal doses of a two per cent. trypan blue solution, given into the jugular vein, resulted always in a generalized tissue stain exclusive of the central nervous system.

Fifteen successful lumbar subarachnoid injections were given in rabbits, cats and dogs, the latter animals offering by far the most satisfactory experimental material inasmuch as their vertebral column allows puncture with greater ease, the prompt appearance of spinal fluid assures the experimenter of the proper position of the needle and withdrawal of the desired quantity of fluid makes room for the injection mass without increasing intraspinal pressure. As much as 10 c.c. of a 1 per cent. solution of trypan blue was injected into the lumbar sac of a large dog without fatal results. In rabbits ½ c.c. of 1 per cent. solution seemed the sublethal maximum. In cats 1 c.c.

We shall first consider the macroscopic and miscroscopic findings in those animals where a sublethal dose was successfully administered. The uniformity of findings in all species of animals used, renders a detailed account of each superfluous.

Macroscopic.—The meninges of the cord were stained a deep blue up to the level of the cervical enlargement, from which point, by gradual gradations, the intensity of the color diminished to the foramen magnum. Above this level the color of the cranial meninges though a distinct deep blue in places, showed many areas where the color was hardly more intense than in the intravascular administration.

The periphery of the cord itself just under the pia was stained a distinct blue for a distance of ½ to 1 mm. below the surface. No such invasion of the brain above the medulla could be distinguished.

Microscopically

Frozen and paraffin sections were made of eight to twenty microns in thickness from various levels of the cord and parts of the brain.

Cord.—The dura and arachnoid of the cord were stained intensely blue. The fine trabeculæ bridging the subarachnoid space were beautifully demonstrated. The pia was similarly stained and its fine trabeculæ extending from the surface into the white substance of the cord were distinctly though faintly blue. Here and there immediately next the pia were seen stained blue cells that presented all the morphological characteristics of true nerve cells but their comparative infrequency precluded proper cytological study and recognition. The ependyma of the cord showed faintly but positively blue.

Brain.—Though the cranial meninges showed macroscopical staining it was not sufficiently intense to permit of recognition in frozen or paraffin sections.

Cases in Which a Lethal Dose Had Been Administered

We believe the findings in these animals, though fatally overdosed, are of experimental value because they demonstrate which tissues of the central nervous system can be reached from a thoroughly permeated subarachnoid space even though it cause the death of the animal to thus permeate this space in *one injection.*

Macroscopically

Cord.—Macroscopically the picture of the cord is the same as in sublethal doses, except for its greater intensity of stain. Instead of the previously described lessening of color at the cervical enlargement and patchy invasion of the cranial meninges the whole central system presents an intense blue color. Section at any level of the cord shows general permeation of the cord substance with blue and in the brain an invasion of its gray or white substance several mms. below the surface.

Microscopic

In addition to the microscopic picture described as occuring with sublethal doses the pial trabeculæ are more distinctly infil-

trated with the dye and throughout the cord and brain substance is seen a fine trabeculated network with here and there a nucleus. This network of cobweblike fibrils corresponds morphologically to the neuroglial framework of nerve tissue and indicates a well-marked general distribution of the dye particularly to the connective tissue elements of the brain and cord.

In the cerebellum, cerebrum and midbrain the stained neuroglial network may be seen for varying distances below the pia. In this reticulated tissue here and there are seen the nerve cells that stained only sparsely with sublethal doses.

In these observations no attempt has been made to limit our report to those tissues which united with the dye in a true intra vitam manner, viz., without causing death or degeneration of the cell, for the sublethal administrations were true intra vitam stains and gave evidence as to the particular tissues stained, whereas the lethally experimented animals served to identify the paths through which the dissemination takes place when either large or repeated dosage may be employed.

Summary

These experimental observations seem to indicate that salvarsan administered subarachnoidally does reach the central nerve tissues more certainly and with greater intensity than when given intravascularly or subcutaneously.

That when administered intraspinally its diffusion to the cranial cavity is less intense than in the cord and that perhaps for intracranial lesions direct injection into the cranial subarachnoid space through a trephine opening would be less dangerous and more effectual.

The writer's thanks are here expressed for the aid rendered him by Professor Frederick Tilney, College of Physicians and Surgeons, New York.

THE ANATOMIC DISTRIBUTION OF CUTANEOUS NEOPLASMS*

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NEW YORK

In determining clinically the variety of any new growth in the skin, a certain amount of consideration may properly be given to the known relative frequency and the known elective site of the different varieties of cutaneous neoplasms. With this fact in mind, it has seemed worth while to note these points while collecting and classifying the skin tumors sent to the laboratory for examination. During the past nine years there have been recorded histologic diagnoses on 352 tumors of the skin, which may be grouped as follows:

Papillomas (including 3 corns and 3 cutaneous horns)		44
Epitheliomas	Papillary ..... 3 Basal cell ..... 57 Squamous 62	122
Pigmented moles		28
Melanocarcinoma		11
Carcinoma metastasis		1
Cyst, implantation		15
Cyst, dermoid		5
Cyst, sebaceous		18
Fibro-epithelial (composite) tumors		3
Keloids		9
Granuloma		7
Lipoma		5
Angioma		22
Fibroma		38
Sarcoma	Fibro .... 11 Angio .... 2 Pure 11	24
Total		352

Many of these specimens were sent to the laboratory merely to add to the collection, but the majority, perhaps, were sent with the idea of determining the character of the tumor; and it is interesting to note

* From the Laboratory of Surgical Pathology, Columbia University.

that 45 per cent. of the whole number showed definite histologic evidence of malignancy.

Epithelioma.—An examination of the anatomic distribution of the epitheliomas shows that 24 were on the forehead, 21 on the cheek, 13 on the nose, 11 on the lip, 9 on the neck, 9 on the hand, 7 on the scalp, 7 on other parts of face or neck, and the rest scattering. About 75 per cent., then, of the epitheliomas were located above the clavicle, and 84 per cent. of all were on uncovered surfaces of the body.

As between the squamous and the basal cell type, two striking differences appear with regard to their chosen sites. For while epitheliomas of forehead, scalp and cheek are quite evenly divided between the two types, all of those on the lip and nearly all of those on the neck (7-2) and hand (8-1) were squamous, but eleven out of the thirteen on the nose were basal cell. Among the other tumors, the total of each variety was so small and the location so diverse that it seemed sufficient to condense the many regions into four broad divisions of Head and Neck, Trunk, Upper Extremity and Lower Extremity.

Papillomas.—The papillomas of this series stand next in number to, and have clinically been confused more often with, epitheliomas than have any of the other tumors.

Of 41 papillomas whose location was given, 20 were on the head and neck. Fourteen (10 of which occupied the hand) were from the upper extremity, 3 from the trunk, and three from the lower extremity — the location of choice was the hand (10). In other words, 49 per cent. were above the clavicles and 75 per cent. in all were on the unclothed surfaces of the body.

Fibromas.—The location of 35 fibromas was given as follows: 11 on the head and neck; 15 on the upper extremity; 2 on the trunk and 7 on the lower extremity. Thus only 31 per cent. were above the clavicle and 40 per cent. on unclothed surfaces.

Sarcoma.—The little series of sarcomas was still more evenly distributed, 5 sarcomas having been found on the head and neck (four of them on the forehead), 6 on the upper extremity, 3 on the trunk and 7 on the lower extremity; 24 per cent. were above the clavicles and 33 per cent. on unclothed surfaces.

Angioma.—The angiomas, on the other hand, show a decided preference for the upper portion of the body, 12 out of 22 coming from the head or neck and 7 from the upper extremity. Of these, 57 per cent. were above the clavicle and 81 per cent. on unclothed surfaces.

Pigmented Moles.—It is difficult to imagine pigmented moles being removed simply as such, and therefore I have grouped them with melanotic carcinomas with which they were most probably confused. Of these two varieties of tumors there were removed, in all, 13 from the head and neck (only one of which was carcinomatous); 5 from the upper extremity (of which, also, 1 was malignant); 8 from the trunk (of which 2 were malignant), and 7 from the lower extremity (5 of which proved to be melanocarcinomas). Four moles and 2 melanocarcinomas were from unknown locations. Only 11 per cent., then, of the melanocarcinomas whose sites were stated were located above the clavicles, and the same percentage were from unclothed surfaces. The far higher percentage of pigmented moles (54 per cent.) removed from unclothed surfaces suggested that some may possibly have been excised for cosmetic reasons.

On combining these results, it appears that, of malignant tumors, the epitheliomas alone show a distinct preference for the head and neck while the sarcomas and melanocarcinomas prefer the trunk and extremities, particularly the lower. Moreover, the epithelio mas are overwhelmingly in favor of unclothed surfaces of the body, while sarcomas and melanocarcinomas generally choose covered parts.

It is interesting to find also that the large majority of epiblastic type of tumors, irrespective of malignancy, occur on unclothed surfaces while those of doubtful or frankly mesoblastic type occur preponderatingly on the covered parts.

812 Park Avenue.

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EXPERIMENTAL MESOTHELIUM

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ELEVEN FIGURES

In a normal animal that survives, repair and sometimes regeneration follows the destruction of tissue. The phenomenon of regeneration, that results in the restoration of structure and function has long been studied and discussed.

Following a physical injury which destroys the free surface cells of the peritoneum or pleura, or the lining cells of blood vessels, two possibilities exist as to how regeneration of the damaged zone proceeds. 1) Cells may grow from the periphery of the given denuded area, taking origin from adjacent, previously existing and intact flat surface cells; or 2) the exposed connective tissue cells of the floor of the injured area may proliferate, be changed in form and become flattened. In the second possibility, the pressure of the opposed surface of the smooth normal peritoneum covering either intestine or other organ is the physical agent, acting continually, that is premised as tending to flatten the surface cells of the damaged area. The pressure and friction of the rushing stream of blood is premised as acting in a similar manner upon the exposed proliferating cells following the destruction of the lining endothelium of a vessel. If there exists a parietal coagulum, reparative cells may invade it and become flattened by the blood stream when the surface of the coagulum is reached. If no coagulum is attached to the denuded area the proliferating cells are immediately exposed to the pressure and friction of the stream of rushing blood. Active friction and pressure between surfaces physically dissimilar, will always result in a mutual change in form until a physical equilibrium is established between them.

The experiments were undertaken in reference to the second possibility; namely, that the exposed deep connective tissue cells making up the floor of the injured area proliferate, change in form and become flattened; thus resulting in the so-called regeneration of a lining membrane in the repair of the surface cells. In other words, an attempt was made to ascertain what change in form takes place in the investing living connective tissue cells in contact for a time with the surface of various, relatively non-irritating, foreign bodies.

EXPERIMENTS

Celloidin is readily moulded into any shape, and after drying may be sterilized by boiling. At the same time celloidin, after the alcohol and ether have evaporated, sets free no chemicals in the dry state when placed in the tissues. Since 1910 more than sixty experiments have been done in which celloidin has been placed in the tissues of several varieties of animals and allowed to remain even as long as one year before removal for study. Its use has always resulted in the formation of a minimum amount of adventitious tissue about the foreign body. After the cellular gathering had disappeared, that is found in and about tissue debris immediately after the operative procedure, neither leucocytes nor so-called 'giant cells' were found in proximity to the celloidin. Celloidin was therefore accepted as relatively ideal non-irritating foreign body for use in operative procedures and experimentations in living tissues.

Experiment No. 1. Celloidin foreign body. Duration 28 days. Dog. Animal No. 33. Operation October 3, 1913. Through a small wound of the skin of the abdominal wall, the subcutaneous tissues were divulsed by thrusting into the aerolar tissue a closed artery forceps, opening the instrument, and withdrawing it while forcibly held open. This injury produced a long, relatively dry tract. Following the introduction of a thin, sterile, smooth sheet of celloidin $2'' \times \frac{1}{2}'' \times \frac{1}{6}''$, the tissues immediately collapsed permitting an easy closure of the skin wound. The wound healed by primary union and the thin strip of celloidin could be felt beneath the skin.

The foreign body together with the adventitious tissue, was removed October 31, 1913, and the specimen (Surg. Path. No. 2642) was fixed in formalin. Paraffin embedding. Sections were cut at

right angles to the free surface. The surface cells, as the section shows, were elongated, closely disposed and parallel to the free surface. Tangential sections were also cut to demonstrate the free surface cells or those in contact with the foreign body, the celloidin strip. The cells were close together and were proven to be flat since the two planes in which the sections were cut were at right angles to each other. Compare figures 2 and 3 with figure 1.

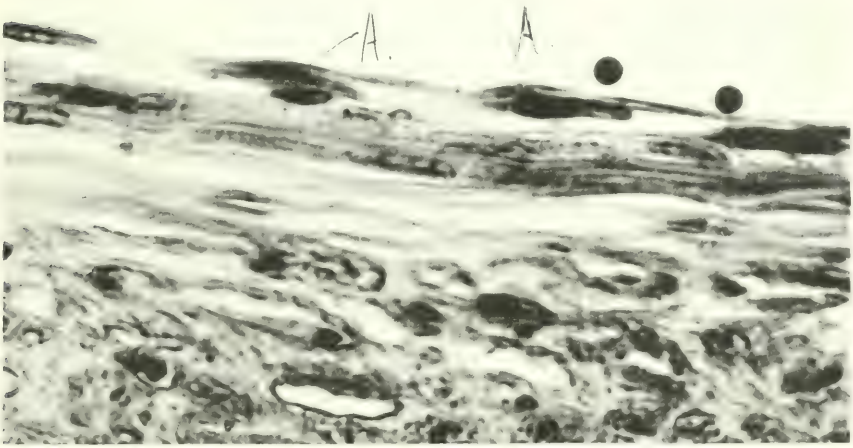

Fig. 1 Photomicrograph, mag. 900. Experiment 28 days. Cross section, showing surface cells in contact with a solid, smooth, flat, foreign body (celloidin). Specimen from subcutaneous tissue of dog No. 2642. See A. A.

Since a histological criterion of mesothelium is a mosaic of black silvered lines, which results when the fresh peritoneum and certain other free surfaces are treated with silver salts, this test was made in the next experiment.

Experiment No. 2. Celloidin foreign body. Duration 21 days. Dog. No. 104. Operation December 5, 1913; a repetition of Experiment No. 1, up to the point of excision of foreign body and adventitious tissue which was done on December 26, 1913. The tissue (Surg. Path. No. 2744) enclosing the celloidin was split open, particular care being taken not to physically injure the specimen. The tissue in contact with the smooth surface of the celloidin was smooth and glistening. A square of the lining surface was placed for one hour in a protargol solution of about 20 per cent. The specimen was then placed in 40 per cent alcohol and exposed to the sunlight. As soon as the

tissue had become a deep brown, frozen sections were cut tangential to the free surface that had been in contact with the celloidin plate. The mosaic of silvered lines in this free surface is shown in figure 4.

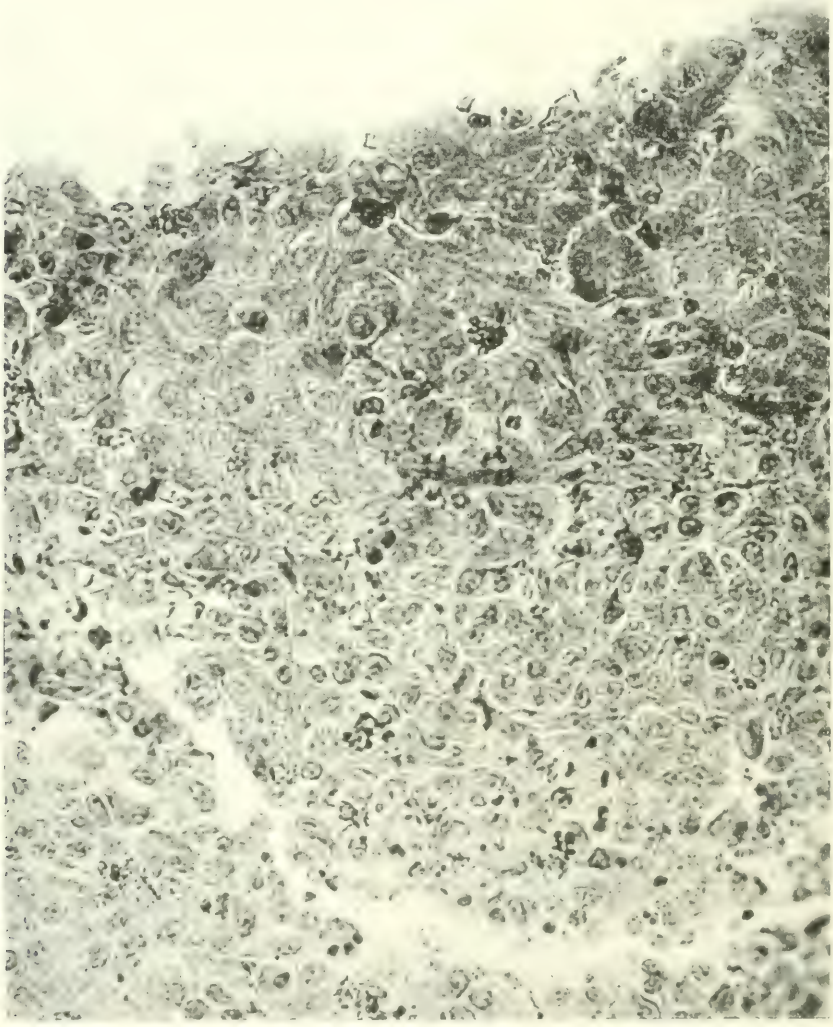

Fig. 2 Mag. 450. Experiment 28 days. Tangential section showing surface cells on the flat in contact with a solid, smooth, foreign body (celloidin). Specimen from subcutaneous tissue of dog No. 2642.

This silver mosaic demonstrated conclusively that the surface is covered by a complete layer of cells, and that the intercellular substance, as far as the silver reaction is concerned, is similar chemically to the

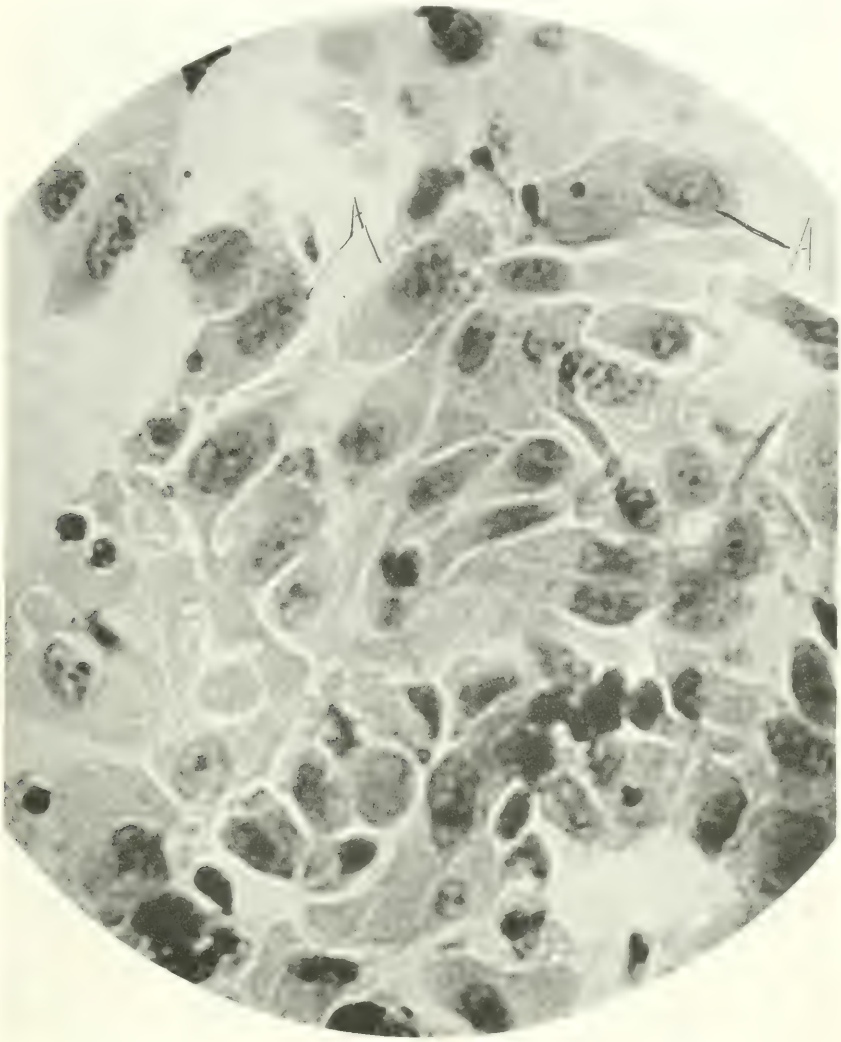

Fig. 3 Mag. 1000. Experiment 28 days. Tangential section showing surface cells on the flat in contact with a solid, smooth, foreign body (celloidin). Specimen from subcutaneous tissue of dog No. 2642.

Fig. 4 Mag. 500. Microphotograph experiment. Silver mosaic in the free surface of tissue in contact with the smooth surface of celloidin. Specimen from the subcutaneous tissue of dog No. 2744. See A.

intercellular substance of the peritoneum, pleura and endothelium of blood vessels.

Paraffin has been used for years to fill cavities in the tissues, and this material forms a solid foreign body, comparatively non-irritating.

Experiment No. 3. Paraffin foreign body. Duration 19 days. Rabbit. Operation December 18, 1911. Melted paraffin was injected through a fine needle into the substance of the cornea. This injection mass spread out in a triangle with the apex at the sclero-corneal junction, the point where the needle had been introduced, and extended outwards over the pupil. The paraffin presented a feathery

appearance in the transparent cornea. The paraffin had been split up into small isolated columns and rod-like shapes when shot into the tissues. These shapes had been preserved when the paraffin solidified. For four days new blood vessels running in from the sclero-corneal junction were seen on the outer margin of the paraffin mass. All these vessels diminished continuously and had disappeared when the cornea was removed, January 6, 1912 (Specimen Surg. Path. No. 1838).

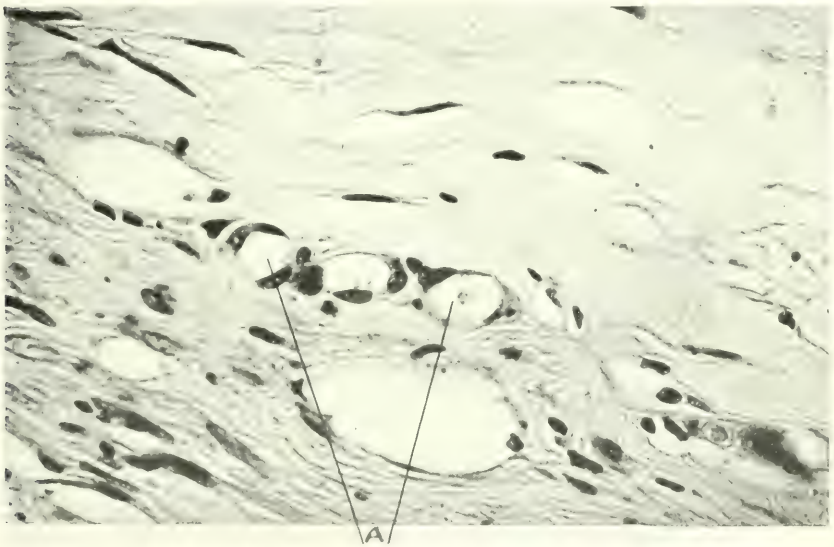

Fig. 5 Photomicrograph, mag. 600. Section of cornea, at pupil. The connective tissue cells (syncytium) surround particles of paraffin. No. 1838. See A.

Microscopic examination: Serial sections through the cornea over the pupil show spaces in the tissue, elongated, oval or round. All these spaces have a distinct outline. They correspond to the situation of the needle-like masses of paraffin in the cornea. Either cells or syncytial masses form definite walls to these spaces (fig. 5). Though the connective tissue cells of the cornea are parallel to its surface, all the cells and syncytial masses bounding the paraffin, conform to the shape of the foreign bodies. In certain of the syncytial masses the nuclei are so numerous and so disposed as to form a structure often noted as a giant cell, of the type known as 'foreign body giant cell.'

This experiment demonstrates that cells become flattened, form syncytium, and adapt themselves to solid foreign bodies; also that giant cells form in tissue purely fibroblastic. Even

though blood vessels appeared in the cornea on the margin of the sclero-corneal junction, microscopically but few capillaries were found in the sections. The left hand opening A, in fig. 5, is undoubtedly a flat syncytium around a paraffin mass.

In addition to the experiments in which solid foreign bodies were used, fluids were also tried. The mucous secretion from the gall bladder, free from bile, seemed fitted for such an experiment. The following experiment, No. 4, was tried, therefore, in order to observe the cells that would line a canal, carrying a bland fluid, constructed through the mesodermal tissues of the abdominal wall.

Experiment No. 4. Foreign body fluid. Duration 28 days. Mucous fistula. Dog. No. 162. Operation December 23, 1912. After the cystic duct had been obliterated between two ligatures, a fistula was established from the gall bladder into and through the abdominal wall to a point in the animal's left flank. This biliary fistula was produced by suturing a rubber tube, $\frac{3}{16}$ inch in diameter, into the gall bladder and permitting it to discharge at the skin opening. At the end of five days the tube was removed. There was a copious discharge of sticky mucous which at times was clear, at other times cloudy.

January 20, 1913, second operation. Two inches of tract were excised (Surg. Path. No. 2289) and fixed in Mann's fluid. Celloidin sections were cut across the tract.

Microscopic examination showed that there was a semi-circular opening in the tissue. The stroma around the canal was exceedingly cellular and contained many small blood vessels. In this stroma, polymorphonuclear leucocytes were numerous, and were grouped about the fistulous tract. Within the tract there were also aggregations of leucocytes. The walls of the fistula were distinct and sharply outlined. The cells of what was certainly the walls of the tract in the living animal were large, oval or elongated, and flattened, with large nuclei.

These cells were so arranged that the long axis was parallel to the wall of the tract. The connective tissue cells beneath the surface were more nearly round and were arranged without reference to the wall of the fistula. The surface was almost exclusively cellular, though at isolated points the wall consisted of a 'ground' or intercellular substance. In places, as shown in figures 6 and 7, the lining was a single layer of cells with elongated nuclei. The tissues of the fistular wall was here actually syncytial in character since no intercellular margins were made out. Fifty serial sections, eight micra in thickness, demonstrated that closely disposed cells lined a larger part of the canal. At points the lining wall was either débris and leucocytes or coagulated exudate, so massed that the actual structure of the wall could not be made out.

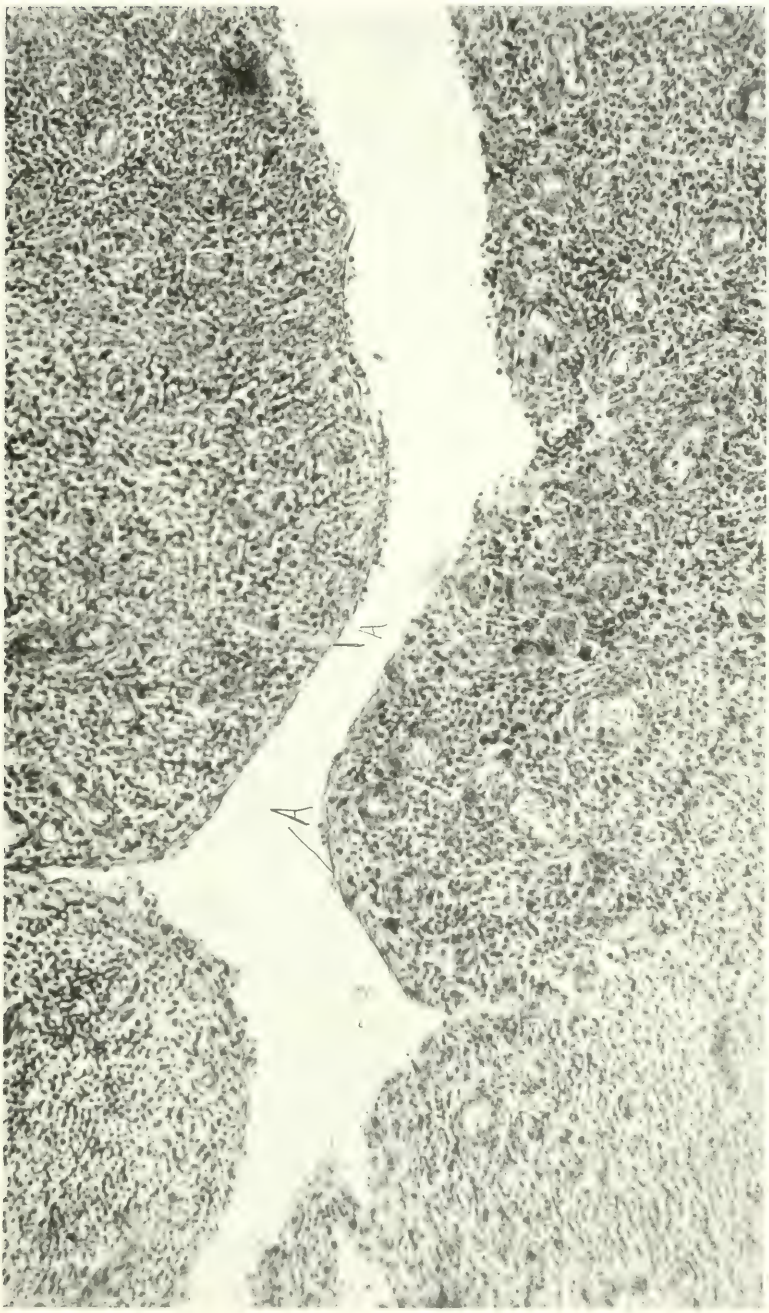

Fig. 6 Photomicrograph, mag. 150. Experiment 28 days. Cross section of walls of mucous fistula showing surface cells. Specimen from subcutaneous tissue of dog. No. 2289. See A. A.

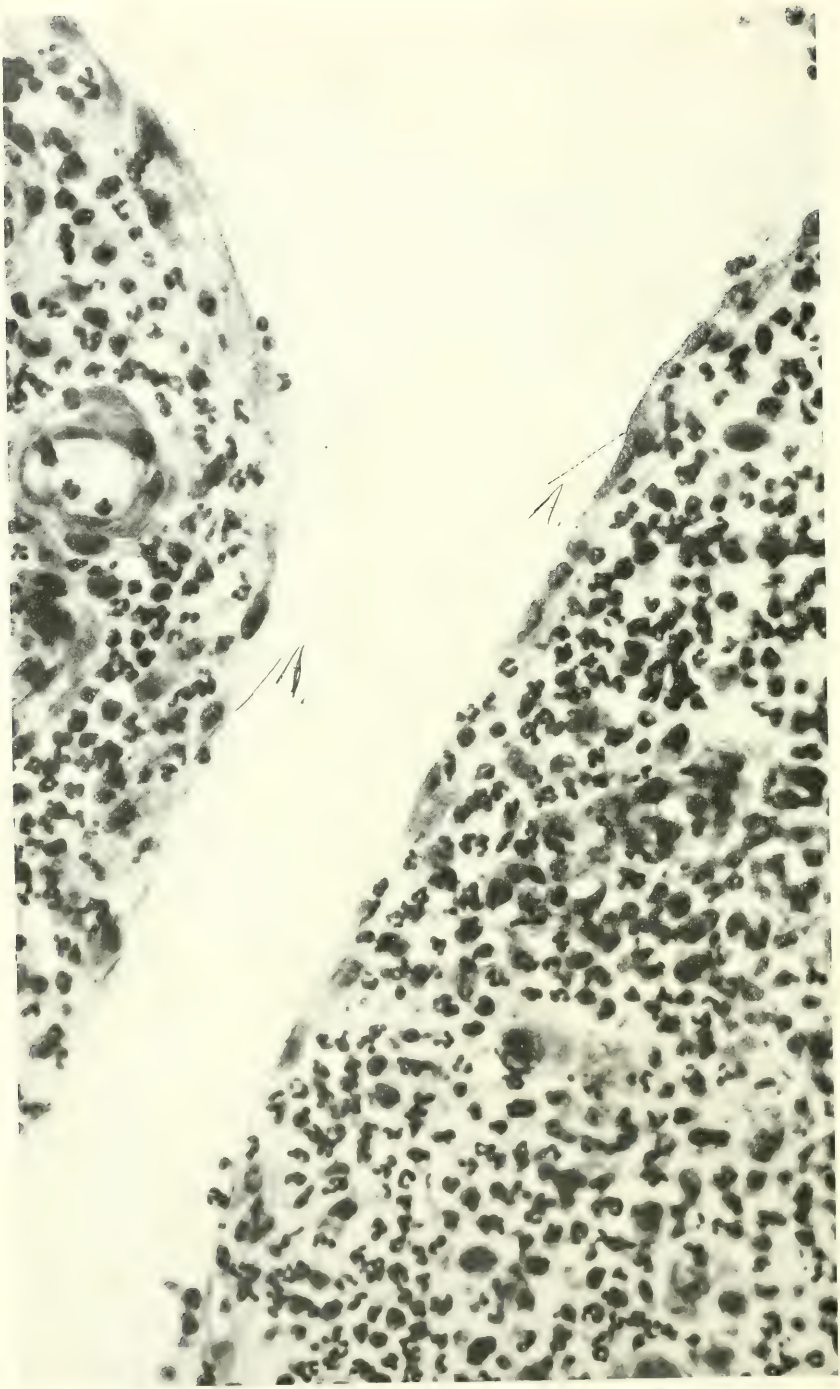

Fig. 7 Mag. 600. Experiment 28 days. Cross section of walls of mucous fistula showing surface cells. Specimen from subcutaneous tissue of dog. No. 2289. See A. A.

In order to obtain a cavity containing serum and not fibrin, 'dead spaces' were studied that frequently obtain where there has been slight inequality in the coaptation of the tissues when operative wounds are closed. Blood clots generally obliterate the greater part of such wound clefts, but the subsequent contraction of the clot and the consequent accumulation of exudate fluid often gives rise to these serum filled spaces. The following operative human case, and experiment No. 4 on a dog, demonstrate such conditions in the tissues, and show the type of lining cells of such cavities.

Case 1. Fluid in contact with tissue seven days. 'Dead space,' Incised wound of thigh. Human adult. Operation by Dr. J. A. Blake, November 21, 1906. Exploratory incision through soft tissues of thigh to the periosteum of the femur. No clinical signs of inflammation developed. Amputation at the hip November 28, 1906, for subperiosteal sarcoma. Specimen (Surg. Path. No. 233) fixed in formalin. Cross section of skin and subcutaneous tissues with exploratory wound revealed a fluid filled space, superficial to deep fascia, about 0.3 cm. in diameter. This space was the result of infolding or puckering of the walls of the wound at the time of the hurried closure. It contained fluid exudate with but little fibrin formation.

Microscopic examination: The wall of the space at points is made up of fibrin and masses of leucocytes. Elsewhere the tissue is thrown into hillocks. The majority of the structural cells are elongated fibroblasts which in numerous places parallel the walls of the dead space. Many of the cells on the surface are elongated fibroblasts, so disposed as to make a definite cellular wall or lining of the space. The stroma beneath the surface consists of large, round, connective tissue cells, with relatively large nuclei at right angles to the plane of the free surface of the space. The actual surface is a layer of syncytium, apparently fibroblastic in character. The nuclei are elongated, stain deeply, are close together in this surface structure, and show no signs of mitotic division. The general appearance of the lining cells is similar to that of the lining cells described in No. 1932 and in No. 2144, shown in figures 8 and 9.

Experiment No. 5. Foreign body: fluid. Duration 7 days. Dog. No. 33. Operation laparotomy, October 2, 1912. Experimental intestinal resection. Uneventful recovery. Autopsy October 9, several hours after death. Gross examination. The abdominal wound was not infected and the healing was by primary union. A section (Surg. Path. No. 2144) across the site of the operative wound demonstrated the irregular but well defined zone leading from the skin through the abdominal wall to the peritoneum, the tract of the healing wound. In the tissues just superficial to the deep fascia there was a cavity or

'dead space' measuring 0.8 by 0.5 cm. and extending along tract of wound. This cavity contained fluid. Specimens were cut from the wall. Paraffin embedding. Microscopic examination. Observation with low power revealed a moderate number of coarse anastomosing strands of fibrin that but partially filled the cavity. Where the fibrin chanced to be attached to the wall of the dead space, the strands extended into the tissues between the cells; as in all healing wounds containing a blood clot. The fibroblasts were directed into the cavity wherever this fibrin served as a support. Where the wall was free from fibrin, sections demonstrated that topographically the surface presented oval or rounded elevations. High power observations of cross sections of the actual surface showed that the cells were large

Fig. 8.

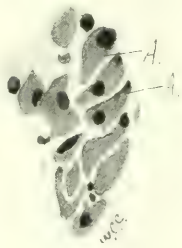

Fig. 9.

Figs. 8 and 9 Duration of experiment 7 days.

Fig. 8 Cross section of wall of 'dead space' showing at A A a complete surface layer of cells.

Fig. 9 Tangential section showing same surface cells, proving that the surface cells are closely apposed and are flattened. See A A.

and elongated, with relatively large, lightly staining nuclei. These cells are tangentially disposed on the free surface of the cavity, and therefore did not reach or point outwards, as did the surface cells at the point where fibrin strands were attached (fig. 8). Sections were cut tangential to the free surface through the wall of the cavity, where it was free from fibrin, to show the surface cells in an opposite plane to that of a cross section. The cells were found closely arranged and varied in shape from oval, elongated, to round; the nuclei were large, elongated and faintly staining, similar to the nuclei of the deeper fibroblasts. Thus it was positively shown that the surface cells were flattened and so disposed as to make a definite cellular covering to the wall (fig. 9). On comparison of a cross section of free surface cells with a tangential section, the cells are seen to be narrower in the plane

parallel to the free surface than in the plane at right angles to the free surface.

The two following experiments are with living tissue grown in the 'observation incubator.' Here it was possible to know absolutely from what tissue the cells were derived that finally enclosed a drop of fluid. In the first experment, the photograph is of the stained tissue, but in the last experiment the tissue is photographed while alive and unstained.

Experiment No. 6. Cultivation of embryonal tissue in vitro. Drs. McWhorter and Whipple. Tissue from chick of 72 hours' incubation. Specimen (Surg. Path. No. 2374), a small fragment of ectoderm and mesoderm was planted in coagulated plasma. Both the epithelium and the connective tissue proliferated, forming the type of structure shown in figures 10 and 11. As the connective tissue grew and advanced, it was observed encircling a vacuole in the plasma. The serum formed this vacuole as the fibrin around contracted. Since there was no fibrin within the vacuole, the proliferating connective tissue cells met with no support, and reacted to the vacuole as does tissue elsewhere in contact with a non-irritating solid or a fluid foreign body. This specimen was stained en masse and photographed.

Experiment No. 7. Cultivation of tumor tissue in vitro. Drs. McWhorter and Whipple. A small fragment of tissue (Surg. Path. No. 1942) from a sarcoma of a St. Bernard dog was planted in coagulated dog plasma. The connective tissue stroma of the tumor proliferated radially. At one point a vacuole in the mass of coagulated plasma was encountered. This vacuole apparently contained serum which had exuded from the coagulum. Figure 11 of the unstained living tissues demonstrates that the cell structure surrounds the vacuole as a syncytium. The large cell in the centre of the vacuole was moving on the surface of the cover slip, and not on the same plane as the wall of the vacuole. The focal distance of the lens at the magnification of 150 times makes it possible to show both the wall of syncytium enclosing the vacuole and the superimposed cell.

In this change in form of the cells that occurs in the tissues when in contact with a 'foreign body' it is probable that the cells are responding to a physical or chemical reaction in progress in the tissues. In substantiation of this belief the statement of Drew[1] may be quoted, who working with pecten maximus experimentally implanted ovary in muscle tissue. He says

[1] Drew, G. Harold. Experimental Metaplasia. Jour. of Exp. Zoöl. No. 10, 1911, p. 349.

Briefly summarised my results show that after implantation of a portion of the ripe ovary into the adductor muscle a layer of fibroblasts is formed around it and coincidently the ovarian tissue is invaded by phagocytes and degenerates. After the lapse of about six days no trace or organized ovarian tissue remains, but there is left a cyst surrounded by fibroblasts, and containing blood corpuscles and a

Fig. 10 Photomicrograph, mag. 150. Plant from chick embryo, in vitro culture. Stained mesenchymal cells surround a vacuole in the coagulated plasma. No. 2374.

quantity of small granules having the orange color of the yolk substance. After the lapse of about 20 days more, the innermost fibroblasts gradually change their shape, and form a layer resembling columnar epithelial cells which later become ciliated. Eventually the whole cyst becomes lined with well defined ciliated epithelium, which persists at least for 120 days, which is the longest period I have yet succeeded in keeping the animals alive in the experimental tanks. I

consider that there is some evidence to show that this change of the fibroblasts into ciliated epithelium is a reaction to the presence of some definite chemical substance within the cyst.

He further believes the fibroblasts of the implanted mass die and that the cyst lining is formed from the fibroblasts of the site of injection. He undertakes experiments to prove that the lining

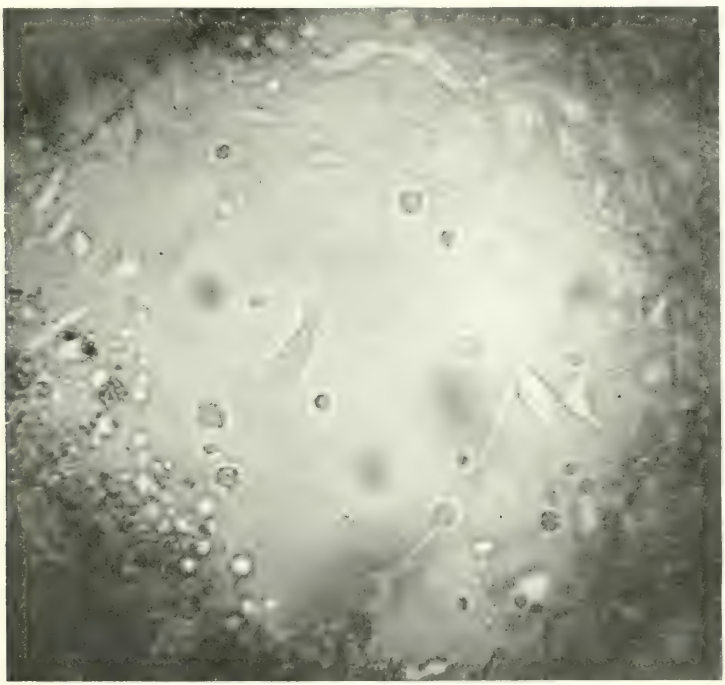

Fig. 11 Photomicrograph, mag. 150. In vitro culture. Unstained growing tissue. The connective tissue cells surround a vacuole that occurred in the culture media. (Coagulated plasma) No. 1942.

cells of the cyst have not originated from the ciliated cells of the transplanted oviduct, but from the cells of the host, at the site of injection.

Drew and DeMorgan[2] performed some experiments in pecten maximus to determine the origin and method of formation of the

[2] Drew, G. Harold and De Morgan, W. The origin and formation of fibrous tissue produced as a reaction to injury in Pecten Maximus. Quart. Jour. Micro. Sci., vol. 55, part 3.

fibrous tissue formed as a capsule or wall about foreign bodies. They believe these structures arise from the fibroblasts in the walls of blood spaces and in the intermuscular connective tissue. The cells divide, migrate and arrange themselves in a concentric manner about the foreign body. These fibroblasts thicken and later present a somewhat stratified appearance. These observers believe the reaction to injury in the animal forms used is exactly similar to that which occurs in vertebrates.

In conclusion it may be stated the subcutaneous connective tissues react to the presence of a smooth surfaced, non-irritating foreign body, i.e., celloidine, in such a manner that there results a distinct pavement layer of flattened cells, the outlines of which are demonstrable by the impregnation of their intercellular substance with a silver salt. The tissues of the cornea, free from blood vessels, in contact with paraffin form similar flattened cells. Cells that are flattened and arranged to form a definite wall, lined the mucous fistula and the 'dead spaces' containing serum. It should be observed that all these results occurred in the connective tissue stroma of the structures injured by the experiment. Drew and DeMorgan obtained a reaction in the connective tissue stroma when foreign bodies had been introduced. This encapsulation of foreign bodies by connective tissue is a well known reaction of the tissues. The experiments with living tissues in the 'observation incubator' showed a similar cellular lining in the vacuoles in the media of coagulated plasma.

The above experiments show after repair is complete that the free surface cells of accidental spaces in the tissues are flattened and form a pavement. In other words after an injury, the connective tissue cells that are exposed become changed in the above manner. Therefore the second hypothesis premised in this article is tenable, namely, that the flat cells of serous surfaces and those lining blood vessels may regenerate from deep connective tissue cells and do not necessarily arise from adjacent intact mesothelial or endothelial cells.

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A TRANSPLANTABLE CHONDRO-OSTEOSARCOMA IN A DOG

BY JOHN E. MCWHORTER
AND
FREDERICK PRIME, JR.
OF NEW YORK

(From the Laboratory of Surgical Pathology, College of Physicians and Surgeons and the George Crocker Special Research Fund, F. C. Wood, M.D., Director, Columbia University)

SPONTANEOUS neoplasms of rodents—particularly of the mouse and rat—have been successfully transferred to other animals of the same breed and are apparently capable of continuous growth for an indeterminate number of generations. Attempts to propagate tumors of other mammals have, for the most part, met with but partial success, owing to the fact that growth of the transplant usually ceases shortly after the transfer. Then regression takes place and the tumor disappears in the first or second generation.

This paper records the successful transplantation, through three generations, of a mammary chondro-osteosarcoma of a dog, in which development of bone was observed in the first generation of transplants.

Clinical History of Dog with Spontaneous Tumor.—On February 23, 1914, a dog, the property of Mr. S., was admitted to the Animal Hospital of the Department of Surgery with the following history:

Breed: Wire-haired fox terrier. Sex: Female. Age: Eleven years. Present history: Eighteen months before admission to the hospital, a small nodular mass was noticed in a mammary gland of the left lateral region. The growth had been extremely slow until a month before admission, when it had rapidly increased in size until it reached such proportions as to interfere with walking. Previous to the period of rapid growth the dog seemed well, but from then on her general condition rapidly deteriorated.

Physical Examination.—The tumor mass was irregular in outline and roughly spherical, measuring about 10 cm. in diameter; it was loosely adherent to the skin, but freely movable on the deeper structures. In the left cervical region a second tumor was found which measured about 4×3 cm., apparently it was not connected with the mammary tumor and was not observed until the period of rapid growth of the latter.

Operation.—The primary tumor was excised with aseptic precautions under cocaine anæsthesia, by Dr. St. John, on February 27, 1914. It was somewhat lobular in form, well encapsulated, and only loosely adherent to the deeper structures. No evidence of infection was present. The dog made a good recovery from the operation and was taken home five days later, but the next day it was

said that she fainted and was chloroformed by her owner. No autopsy was obtained.

Description of Primary Tumor.—Macroscopic appearance (Surgical Pathology No. 2884, Fig. 1): The specimen consists of a well-encapsulated, lobulated mass of tissue, roughly spherical in outline, measuring 9.5 × 10 cm. The capsule is rather thick and very dense. On cross-section the entire mass is firm and very dense, some areas cutting like cartilage, while others are of bony consistency. The cut surface presents a variegated appearance, suggesting that the tumor is made up of many types of tissue. Some areas show dilated spaces filled with an amorphous substance, others a mass of tissue projecting from the wall into the lumen, still other regions, because of their hard consistency and gritty feel, suggest spongy bone. Scattered here and there through the structureless tissue of the stroma are small areas which resemble cartilage in appearance and density.

Microscopic Examination (Figs. 2 and 3).—Sections show the glandular tissue in some areas to be compressed, the epithelial cells hypertrophied, and for the most part vacuolated. Many of the acini show a heaping up of these cells, with the production of a papillary outgrowth from the walls into the lumina; this cellular arrangement is orderly and shows no tendency to infiltration. In other areas the acini are widely dilated and contain an amorphous substance, staining deeply with hematoxylin. These glandular areas are generally surrounded by zones of round-cell infiltration. Extending outward beyond the mammary gland and abutting upon the lobules of the breast is a mass of dense connective tissue which becomes denser where it meets the intralobular septa. Beyond this margin the tissue is more cellular and is of a much looser structure.

These cells, for the most part spindle-shaped, are rapidly proliferating, as evidenced by their intense staining and the number of mitotic figures. This mass of connective tissue in some areas becomes less cellular and long fibrous strands project from it, interweaving with other strands, to form zones of dense fibrous tissue. The cells lying between these strands have lost their spindle shape and become stellate. This tissue gradually merges into fibrocartilage.

Other sections show the connective-tissue cells to be more rounded than oval; more of the oat than the spindle shape. In many of these areas are scattered nodules of spongy bone.

The formation of bone seems to be one of the characteristics of this tumor; it is scattered generally throughout the connective-tissue stroma; but neither the type nor the arrangement of these connective-tissue cells seems to have any definite influence on its formation. The chief elements of this neoplasm therefore are: (1) Sarcomatous tissue; (2) spongy bone; (3) cartilage.

Transplantation of Tumor.—When the tumor was excised several bits of tissue were taken at random from the mass for transplantation.

The technic of transplantations was in all cases the same. The tumor was placed in a sterile dish, and thin slices were cut through the apparently malignant portions. Fragments from these areas were cut into pieces about 0.02 Gm. in weight, and were introduced through an inoculating needle subcutaneously into the axillary regions.

The original tumor was inoculated into both axillary regions of twenty dogs, five of which were part terrier, and not of exactly the same breed as the original dog. Four of the five were puppies and were inoculated on the day of birth. One month later it was found that five of these twenty dogs had developed tumors,

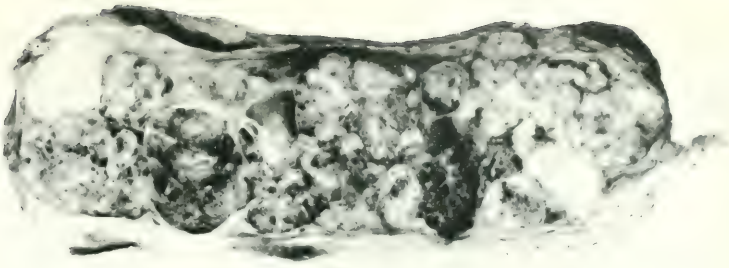


Fig. 1.—Surgical Pathology No. 2884. Gross section of primary tumor.

Fig. 2.—Surgical Pathology No. 2884. Primary tumor showing distribution of carcinoma, sarcomatous tissue and glandular structure.

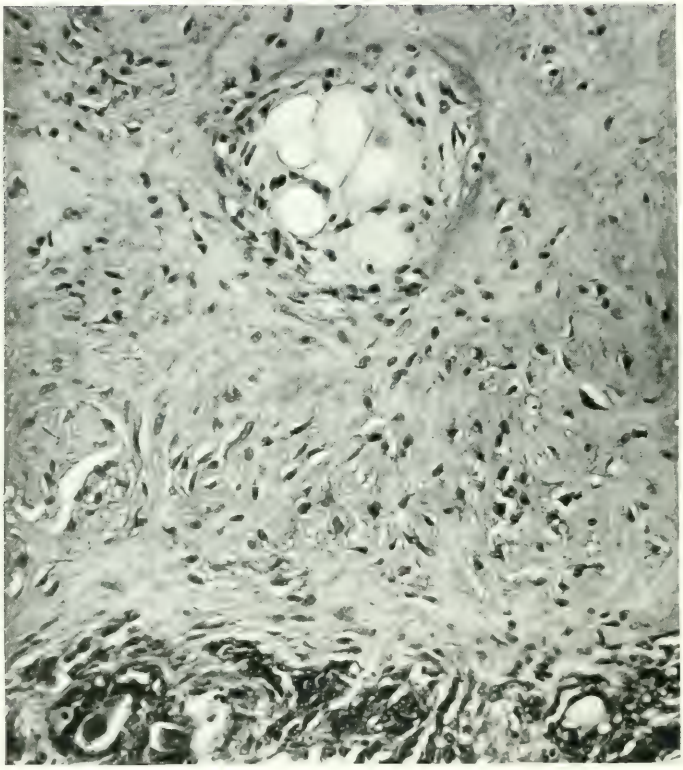


FIG. 3.—Surgical Pathology No. 2884. Primary tumor showing junction between cartilage and hyperplastic glandular tissue.

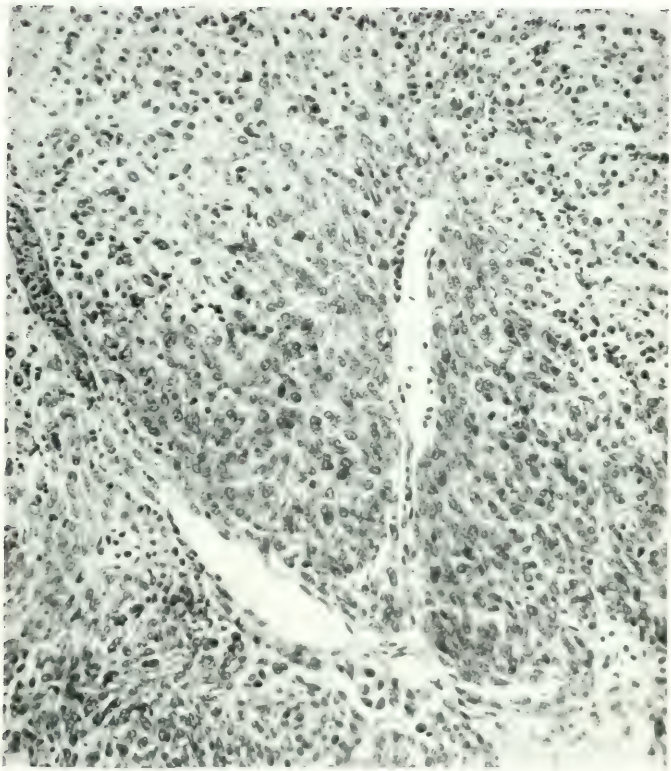


FIG. 4.—Surgical Pathology No. 2956. Tumor showing necrosis of carcinomatous tissue [illegible] in neighborhood of capillaries.

Fig. . —Surgical Pathology No. 2053. Section showing formation of bone in 'transplant'.

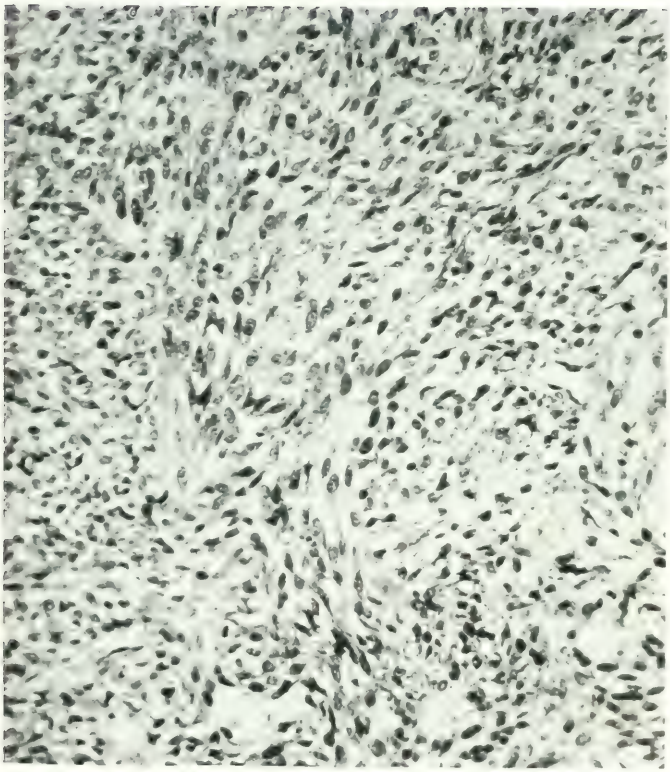


Fig. 4.—Surgical Pathology No. 2015. Section showing [illegible]

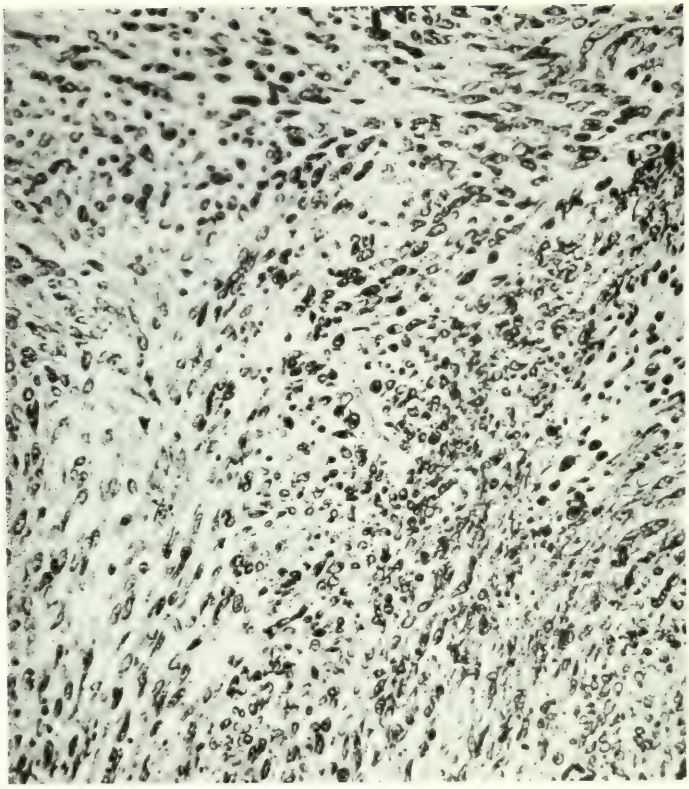


FIG. 7.—Surgical Pathology No. 2978. Section showing cellular arrangement in second generation of transplant.

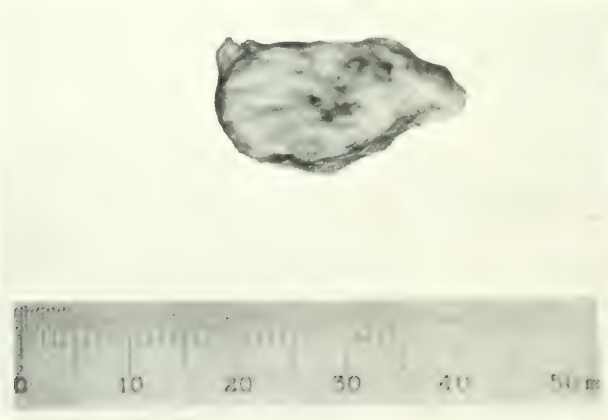


FIG. 8.—Surgical Pathology No. 3000. Gross appearance of transplant from second generation.

two of which had been inoculated on the day of birth (cf. Chart I). Of these there were three females and one male, while in one the sex was not recorded. One dog of this group died before the tumor was removed. From a second dog, an adult female, the tumor was removed and inoculated in both axillary regions of twenty dogs. At the same time an attempt was made to grow some of this tissue in plasma, but without success. A week later the tumor in a third dog had disappeared.

The growth in one of the two puppies, however, had increased enormously, measuring 3.1 × 2.7 cm. This was removed thirty-nine days after inoculation and transplanted into both axillæ of six other dogs. The second puppy with a tumor died at the end of forty-four days, and as the tumor was decreasing somewhat in size it was not transplanted into other dogs.

In the second series of the second generation (cf. Chart II) the transplants at the end of seven days showed growth in five out of six dogs; in three of these the tumor appeared on both sides. Four of these animals were males and one was a female. These tumors were not as large as those in the previous generation, nor did they grow as rapidly. At the end of a month all but one had disappeared. One was inoculated at the end of eleven days into seven young dogs but did not proliferate in the new hosts.

The first series of the second generation of transplants (cf. Chart III), showed at the end of three weeks tumors in three female dogs out of twenty inoculated, one of which had nodules on both sides. Seven days later another dog of this series had developed a growth, and seven days after this the last tumor of this series was found in a male dog. One of the neoplasms from the dog which had developed a growth on both sides was inoculated with negative results into both axillary regions of twelve dogs. The tumor on the other side was left *in situ* and disappeared at the end of another month. Another tumor of this series which was beginning to retrogress was partially removed and inoculated without success into both axillæ of ten dogs. The portion left in the host soon disappeared.

Under the existing conditions, three months after the tumor had been removed from the original host, the tumor strain died out. During this period it was carried by transplantation through three generations.

Description of Transplanted Tumors.—Of the first generation of transplants, three tumors have been selected for description, as they fairly represent all the growths in this series. Surgical Pathology Nos. 2963 and 2956 were growths in puppies one day old, the results of transplants from the original host. These puppies, though not of the same breed as the original dog, were, however, closely related, as their mother was partly terrier.

Surgical Pathology No. 2545, a tumor growth in an adult dog, planted with the same tissue and at the same time as 2963 and 2956. This animal was not a true terrier, although it undoubtedly had some terrier blood. Tumor 2956 was removed thirty-eight days, 2963 forty-four days, and 2945 thirty-one days after transplantation.

Surgical Pathology No. 2956. Macroscopical appearance. The specimen consists of a well-encapsulated mass somewhat lobulated and oval in outline, measuring 5.5 × 3 cm. On section it is found to be made up of dense tissue that extends from the surface inwards for about 1 cm. The centre consists of a soft friable mass, probably degenerative tissue; elsewhere as the knife passes through the tumor a number of areas are encountered which have a hard gritty feel.

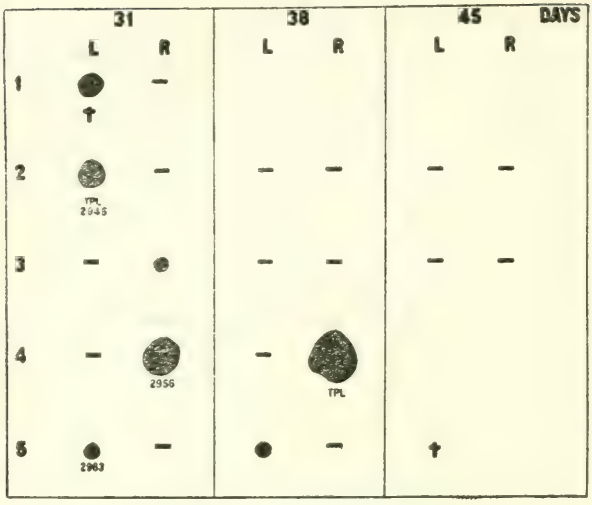


CHART I.—First generation of transplants.

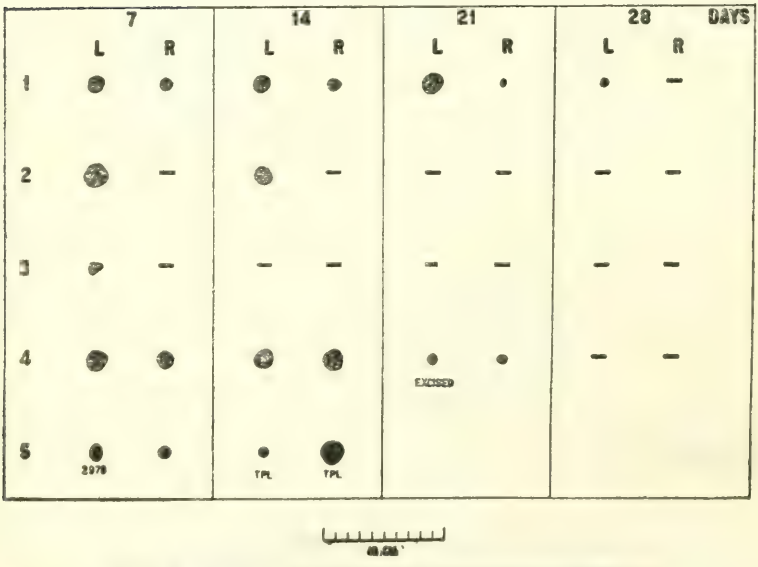


CHART II.—Second series of the second generation of transplants.

Microscopical Examination (Fig 4).—Sections show the tumor to be only partly encapsulated. The capsule is made up of a thin layer of connective tissue containing many newly-formed blood-vessels. The stroma of the tumor is composed chiefly of spindle-shaped connective-tissue cells which are densely

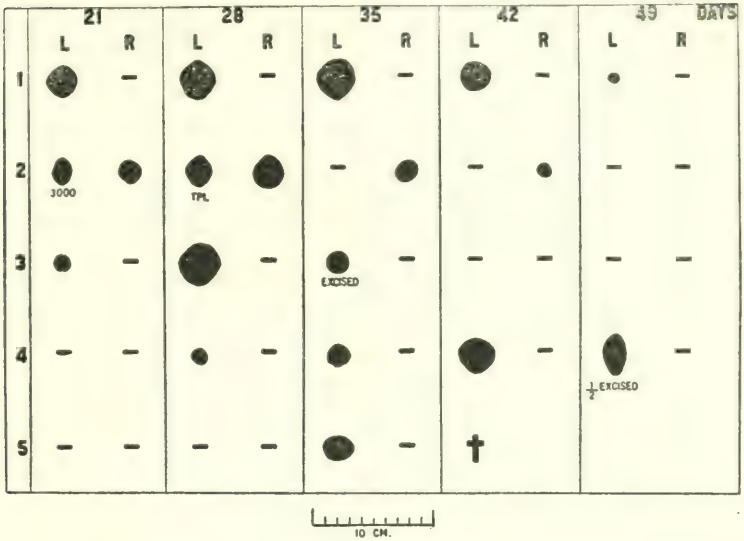
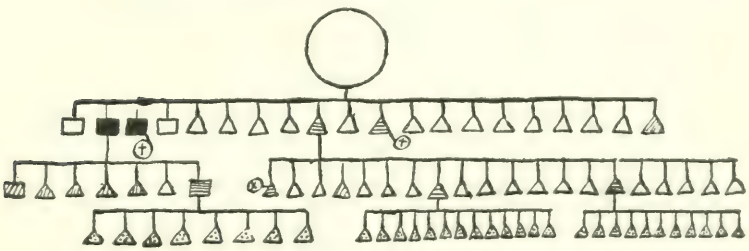


CHART III.—First series of the second generation of transplants.



Pedigree of Transplanted Tumor.

Primary tumor of original host.

Puppies transplanted with tumors.

Adult dogs transplanted with tumors.

A progressive tumor

A regressive tumor.

Dogs in which the tumor strain died out. The exact number of takes in this series was not recorded, as the intial growth soon gave way to regression and complete disappearance.

Animal died.

packed together near the surface, and are apparently growing very rapidly. Toward the centre the cells are much more loosely woven and are more round than spindle; and by their intense staining show evidence of high activity. The centre is made up of broken-down cells, lymphocytes, red blood-cells, and fibrin. Scattered at irregular intervals throughout the tumor and situated far beneath the surface are areas of spongy bone. The tumor as a whole shows many newly-formed blood-vessels throughout the stroma.

Surgical Pathology No. 2963. Macroscopical appearance. Specimen consists of an oblong tumor mass, measuring 2×1 cm., regular in outline, and well encapsulated. On section the tissue is dense, cuts with difficulty and has a decidedly gritty feel.

Microscopical Examination (Fig. 5).—Sections show the tumor to be well-encapsulated. The thick capsule of the tumor is made up of dense connective tissue. Beneath the capsule are areas of calcification, from which strands of very densely packed connective tissue extend inward toward the centre of the tumor. As they approach the centre, the strands become less dense and finally merge into a loosely scattered mass of connective-tissue cells. This picture shows beautifully the transition between the loosely scattered connective-tissue cell and spongy bone.

Surgical Pathology No. 2945. Macroscopical appearance. Specimen consists of a small partially encapsulated tumor mass measuring 1.5×0.75 cm. On section it is firm, cuts easily and apparently is of the same consistency throughout.

Microscopical Examination (Fig. 6).—Sections show tumor to be only partially surrounded by a capsule consisting of a thin band of connective tissue, the stroma being formed of connective-tissue cells of the spindle type. Toward the centre of the tumor there is a small circumscribed area consisting of degenerated cells. The tumor infiltrates the surrounding tissue through breaks in the capsule, and its growth is very active as may be seen from the number of mitotic figures. This tumor is moderately vascular. There are a few vessels in the stroma, but the greater number are situated near the periphery.

Second Generation of Tumors.—The tumors developing from transplants taken, at the time of excision, from 2956 and 2945, were selected for description because they are typical of this series.

Surgical Pathology No. 2978. Macroscopical appearance. This growth, the result of plants taken from 2956, is an encapsulated mass measuring 1.65×0.6 cm. On section it is found to be firm and of homogeneous consistency throughout.

Microscopical Examination (Fig. 7).—Section shows tumor to be surrounded by a capsule of dense connective tissue. The stroma consists of a densely-packed mass of spindle-shaped connective-tissue cells showing a uniform arrangement throughout the whole extent of the tumor. The large blood-vessels are confined to the capsule; the stroma is apparently without very abundant blood supply. Although the tumor from which this plant was taken developed bone, in this generation all evidence of it is lacking. The conditions which made possible the development of bone in the tumor of the first generation were apparently the same in the second, with one possible exception, namely, the puppies were considerably older than those of the first generation.

This series then resembles in its growth all the transplanted tumors with the exception of the transplants in two puppies in which bone developed.

Surgical Pathology No. 3000 (Fig. 8). Macroscopical appearance. This

tumor, typical of this group, a transplant from 2945, consists of an encapsulated, irregular, oblong mass of tissue measuring 2.3 × 1.2 cm. On section it is found to be of firm consistency, excepting toward the centre, where it is soft and friable.

Microscopical Examination.—Sections show the tumor encapsulated by a thin layer of connective tissue. At one point the capsule has been broken through and the neighboring tissue is infiltrated by the tumor cells. The tumor is made up of irregularly arranged masses of spindle-shaped connective-tissue cells densely packed at the periphery, and much looser as the centre is approached. The centre consists of a homogeneous mass of broken-down cells. This area is completely surrounded by round cells. The tumor is only very moderately vascular; the majority of the vessels are confined to the capsule.

Third Generation.—In this generation the tumor strain died out. The transplants were made indiscriminately in adult dogs because of a lack of puppies and dogs of proper breed. Although the majority of these grafts underwent an initial growth, the limit of their proliferative capacity was reached in producing in some cases nodules 1 cm. in diameter. It was remarkable with what rapidity these tumors disappeared, for one measuring more than 1 cm. in diameter was not palpable at the end of a week. The microscopic picture of a regressing tumor may be briefly described as usually consisting of a rim of living tumor cells completely infiltrated with round cells, and having for its centre a homogeneous mass of broken-down cells.

SUMMARY AND CONCLUSIONS

1. A mammary chondro-osteosarcoma arising spontaneously in a dog has been transplanted into other dogs through three generations, covering a period of ninety days.

2. By far the most successful growths took place in very young puppies and particularly in those of nearly the same breed as that in which the original tumor grew.

3. The tumor showing bone as found in the puppies of the first generation more nearly resembled the original tumor than did the growths in adult dogs.

Therefore it is not unreasonable to assume that by using very young puppies of the right breed, a tumor strain might be developed similar to that already established in rats and mice.

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THE INEFFICACY OF PYLORIC EXCLUSION BY FASCIAL BANDS*

AN EXPERIMENTAL STUDY

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of New York City

(From the Department of Surgical Research, College of Physicians and Surgeons, Columbia University)

When it was shown that exclusion of the pylorus by the silk ligature method resulted in incomplete or too temporary closure,[1] attempts were made to occlude the pylorus by other materials. Of these, fascia offered the most promising possibilities, for it is a tissue of great resistance and remains viable, when transplanted, for a considerable period.[2] In 1908, Bogoljubow [3] reported that he had obtained, experimentally, complete occlusion of the pylorus by ligation with fascia strips. The operation was performed on the human being by Wilms [4] in 1911; subsequently by a number of other surgeons. Uniformly successful results were reported. The procedure bid fair to become the standard one for pyloric exclusion, and a difficult problem in gastric surgery appeared solved. Recently, however, a very few unsatisfactory results—*i.e.*, perigastric adhesions or patent pylorus—have been described. It thus became a question of interest to determine definitely if the experimental basis for the operation was entirely correct.

In analyzing the experimental studies that have been made, one finds that, in the work of Bogoljubow, the longest period of observation after operation was fifty days. That period was not appreciably longer in Protassoff's [5] experiments, which substantiated the findings of the originator of the method. Von Tappeiner [6] investigated the great variety of exclusions that have been advocated and found that the fascial

* Read at the December, 1915, meeting of the Surgical Section of the New York Academy of Medicine.

[1] At the present time there is no general agreement upon the desirability of complete or permanent closure, or even upon the indications for exclusion. Nevertheless, it is important to know what results may be expected after the application of any of the proposed and practised methods.

[2] The question of permanent viability is as yet unsettled, and is not considered in this paper.

[3] Bogoljubow: Arch. f. klin. Chir., 1908, vol. lxxxv.

[4] Wilms: Deut. med. Wochen., 1912, No. 3.

[5] Protassoff: Tenth Russian Medical Congress, 1910.

[6] Von Tappeiner: Bruns Beitr., 1912, vol. lxxx.

band method was the one satisfactory procedure. However, he performed only three exclusions by that method. His longest period of observation was two months. At the post-mortem examinations water escaped from the pylorus, drop by drop, in every instance. Very recently Gibson and Beekman [1] have also reported a series of experiments dealing with various methods of pyloric exclusion. They strongly favor the fascia ring method for the human being, yet in only one of their seven experiments (observations were made as long as three months after operation) was a "functionally complete" occlusion obtained; in the remaining experiments the occlusion was termed "incomplete." The authors concluded that "there was undoubtedly some stenosis, and in all probability only a small amount of stomach contents passed through, the greater part going through the gastrojejunal anastomosis."

The details of technic of the experiments I performed need not be described. It will suffice to mention the two methods employed. Either the pylorus was tightly ligated with a strip of fascia lata knotted about it, or the fascia, as a broad band, was sutured snugly about the pylorus and infolded by two or three tiers of sutures. No-loop gastroenterostomies, with large stomata, were made. Table I summarizes

TABLE I

Dog No.	Period after operation at which examination was made	Condition of pylorus
135	5 days	Opened with maximal hydrostatic pressure.
172	6 days	Water-tight.
131	1 week	Opened with considerable pressure.
157	2 weeks	Patent.
145	3 weeks	Opened with moderate pressure.
303	3 weeks	Opened with considerable pressure.
167	1 month	Patent.
118	2 months	Patent.
104	2 months	Opened with moderate pressure.
97	6 weeks	Patent.
82	3 months	Opened with slight pressure.
331	5 months	Patent.
341	5 months	Patent.

my results. Only once (Dog 172), at an examination made six days after operation, was the pylorus found completely water-tight. When the examinations were made at the end of one to three weeks it was usually found closed until the hydrostatic pressure within the stomach became high. Even in this period, however, the pylorus was found open in one experiment (Dog 157). It is of interest to note that the cause

[1] Gibson and Beekman: Ann. of Surg., 1915, vol. lxi.

for this (partial) exclusion observed shortly after operation appeared to lie in extensive peripyloric adhesions that generally developed, rather than in the constriction made by the band. In microscopic sections the fascia was found adherent to the gastric wall, but was evidently stretched, despite its great tensile strength, by the underlying musculature.

When post-mortem examinations were made one to three months after operation the pylorus either opened with moderate hydrostatic pressure or was quite patent. In the latter instance the fascial band was incorporated with the gastric wall, but it made a palpable ring that

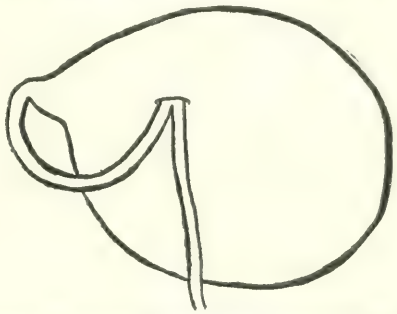

FIG. 1.—Extreme dilatation and rotation of the stomach after incomplete occlusion of the pylorus.

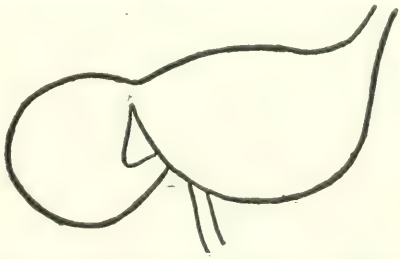

FIG. 2.—Dilatation of the duodenum after incomplete occlusion of the pylorus.

fitted loosely about the pyloric muscle. This was evident, too, in the microscopic sections. That the pylorus was widely open was certainly unquestionable at the post-mortem examinations five months after operation. In short, the error in the work of Bogoljubow and his followers was the brief period of observation after operation. Only very temporary pyloric exclusion results from ligation with fascial bands and even this cannot be expected to develop regularly.

A question that naturally arose was: Would pyloric exclusion by fascial bands be successful if the pylorus was first crushed? Several experiments were made to determine the point. In one, at the post-mortem examination two months after operation, the pylorus remained

closed with moderate, but opened with considerable, hydrostatic pressure. It was found patent, without water pressure, in the remaining experiments. Indeed, it was quite open in one of these experiments despite the fact that a large inflammatory tumor, closely surrounding the pylorus, had developed around some gauze sponges left near the stomach.

As a last effort to establish efficient pyloric exclusion with fascial strips the following operation was performed a few times: The stomach was opened near the pylorus, gauze sponges or strong tincture of iodine were vigorously rubbed into the pyloric mucous membrane, the gastric incision was closed, and the constricting fascial band then applied. These experiments were as unsuccessful as the previous ones, therefore the results need not be detailed.

Before closing, I wish to call attention to two deaths in the experimental series. They are presented because they appear to be directly ascribable to disturbed mechanical arrangements, the sequel of incomplete pyloric occlusion. Both animals were extremely emaciated and, at the post-mortem examinations, complete or almost complete retention of gastric contents was evident. In one (death one month after operation) there was enormous gastric dilatation and anterior rotation of the stomach (Fig. 1). The intestinal tract was quite empty. When the stomach was rotated backward gastric contents escaped into the duodenum through the pylorus and into the intestine at the stoma. The second animal died five months after operation. The stomach was markedly dilated, the pylorus was somewhat narrowed, and there was a tremendous dilatation of the first part of the duodenum (Fig. 2).

CONCLUSIONS

1. Experimental evidence of pyloric exclusion by fascial bands is incorrect, observations having been made too soon after operation.

2. Permanent pyloric occlusion does not follow experimental ligation with fascial bands.

3. Temporary pyloric occlusion is uncertain after experimental ligation with fascial bands.

4. Experimental pyloric occlusion by fascial bands is likewise unsuccessful after crushing the pylorus or after application of irritants to the pyloric mucosa.

5. Experimental pyloric ligation by fascial bands may be fatal, apparently from disturbed mechanical arrangements following incomplete occlusion.

6. The clinical application of pyloric exclusion by fascial bands therefore rests on an unjustifiable experimental basis.

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A Preliminary Report on a Simple and Rapid Method of Pyloric Closure in Gastro-Enterostomy

BY

GEORGE EMERSON BREWER

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PYLORIC EXCLUSION

An Experimental and Clinical Study[1]

By RICHARD LEWISOHN, M.D., New York City

UNTIL a few years ago gastro-enterostomy was generally considered the method of choice for the treatment of pyloric and duodenal ulcers. It was considered unnecessary to add anything to this procedure. Even in the recent literature we find papers advocating this operation for ulcers of the stomach and duodenum. Paterson, for instance, read a paper before the Clinical Congress of Surgeons, in Chicago, two years ago, in which he stated that "the occlusion of the pylorus is an unnecessary complication of gastrojejunostomy and is based on erroneous pathology."

This opinion, however, is rather an isolated one. In fact most surgeons agree that a simple gastrojejunostomy will not permanently cure the disease. Those who advocated simple gastro-enterostomy for the cure of pyloric and duodenal ulcers, assumed that all the food with the gastric juices would pass directly through the anastomosis between stomach and jejunum, and no longer through the pylorus and duodenum.

Kelling, however, proved conclusively about fifteen years ago the fallacy of this idea. He established a gastrojejunostomy and a duodenal fistula in dogs, and observed that most of the stomach contents were discharged through the duodenal fistula and that only a small amount of food passed through the anastomosis into the intestines. It is thus clear that a simple gastro-enterostomy will not prevent the food from passing over the ulcerated area. To be sure, the regurgitation

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of bile through the anastomosis probably plays a certain curative rôle, by counteracting the hyperacidity of the stomach contents. This alkalinization alone, however, does not suffice to cure ulcers permanently.

Furthermore, it is a well-known fact that without simultaneous resection or occlusion of the pylorus a simple gastro-enterostomy is apt to contract in a very short time. Thus all the food will again pass through the pylorus and conditions which existed before the operation, will be re-established.

The vast majority of surgeons, therefore, agree that pyloric exclusion ought to be added to gastrojejunostomy to insure the permanent cure of pyloric and duodenal ulcers. This consensus of opinion, however, holds good only for the principle of pyloric exclusion. There still exists a great difference of opinion as to the best method of accomplishing this, and in consequence many different methods are in use at the present day.

The different methods of pyloric exclusion can be divided into five groups:

1. Unilateral pyloric exclusion (Eiselsberg).
2. Submucous plastic (Girard).
3. Infolding method (Kelling-Mayo).
4. Exclusion method with the aid of autoplastic material (Wilms).
5. Exclusion methods with the aid of foreign material (suture, etc.) (Kelling-Berg-Cackovic, Parlavecchio, Biondi).

The unilateral pyloric exclusion (Fig. 1*A*) has quite a unique position among all these different methods. For it is universally conceded, that Eiselsberg's method provides for a permanent occlusion of the pylorus. An exceptional case, as the most interesting case of Gerster, reported by Moschcowitz, does not depreciate the value of this method. However, all the other methods of excluding the pylorus, though heralded by their originators as permanent occlusion methods, have not been able to stand the experimental and clinical tests of other investigators, in so far as the question of permanent occlusion of the

pylorus is concerned. Yet Eiselsberg's method is used and justly, only by a comparatively small number of surgeons. For this method is a much more formidable surgical procedure than any of the other methods. And though its absolute ultimate success must be granted, the comparatively great operative risk stands in the way of its general use for the treatment of pyloric and duodenal ulcer.

Girard's method is a reverse Heinecke-Mickulicz pyloroplasty applied to the seromuscularis, but leaving the mucosa intact. A transverse incision is made on the anterior surface of the prepyloric part of the stomach and the walls of the incision united in the longitudinal direction, thus producing a fold of the mucosa, which obstructs the lumen of the pylorus. This method does not seem to have gained popularity, probably because the stitch and band methods obtain the same object in a much more simple way.

The infolding stitch method (Kelling-Mayo) is shown in Fig. 1*B*, and the very simple technique does not require any further explanation. Tappeiner, who tested the different methods of pyloric exclusion in a series of animal experiments, showed that this method does not yield any permanent results. Furthermore, Moschcowitz and Wilensky have lately reported a case, in which they had occasion to test the permeability of the pylorus two weeks after the performance of a gastro-enterostomy, combined with the Mayo exclusion. The patient had to be re-operated upon because of intestinal obstruction. The post-mortem examination showed the pylorus to be patent, "no complete closure of the lumen of the pylorus had been obtained."

Wilms' method of pyloric exclusion (Fig. 1*C*) makes use of a free transplant of fascia (usually taken from the fascia lata) which is used as a constricting band around the pylorus. Kolb, an assistant of Wilms, has reported that this method has stood the test of animal experiments and clinical experience, especially in reference to the permanency of the exclu-

sion. Baggio, however, found that the transplanted fascia was macroscopically and microscopically partly absorbed and necrotic, and the result by no means ideal.

I wish to discuss more in detail the last group, i.e., exclusion with the aid of sutures, wires, cotton tape, etc., because the experimental work, which I am going to describe in this paper, falls under this heading. Three different methods belong to this group: Kelling-Berg-Cackovic, Parlavecchio, Biondi. Though the methods fall in one group in that foreign material is used in all of them for the constriction of the pylorus, the technique differs widely.

Exclusion of the pylorus with the aid of a ligature (Fig. 1*D*) is not a new procedure. It was used by Kelling in animal surgery in 1899, and introduced into clinical surgery simultaneously and independently by Berg and Cackovic in 1903. A double Pagenstecher linen suture, armed with a needle, is carried around the posterior stomach-wall, and is held in place by taking several bites in the anterior wall of the stomach. The suture is then tied and the pylorus thus occluded; the knot is buried by a few single stitches (Berg). This exclusion stitch has acquired great popularity in the treatment of acute and chronic ulcers of the pylorus and duodenum.

Parlavecchio's method cannot be considered as a new one, but simply represents a slight modification of the method just described, in that he substituted a cotton tape for the Pagenstecher stitch. Randisi and Dominici have published excellent results with this method. Leriche, however, has reported that his results with this method were by no means satisfactory.

Biondi's method (Fig. 1*E*) was described in detail by Porta. A longitudinal incision is made across the pylorus through the serosa and muscularis, and the musculoserosa coat peeled away from the mucosa. The mucosa is then cut between two ligatures which have been tied around the tube of the mucosa at

both ends of the incision. The stumps are carbolized and the seromuscularis incision closed with a few sutures.

The Biondi method undoubtedly stands midway between the very simple constriction methods (Mayo, Wilms, Berg, Cackovic) and Eiselsberg's unilateral exclusion. A perusal of the literature shows that none of the simple constriction methods assures permanent results. Eiselsberg's method, as already mentioned, is too great a surgical procedure. Biondi's method would meet all requirements, if the technique were as easy of performance as the constriction methods and as certain of permanency as the method of Eiselsberg.

It is obvious though that the method as described by Biondi can not assure a permanent closure of the pylorus. The ligature around the mucosa stumps is apt to cut through at an early date. Furthermore, the regenerative power of the mucous membrane is so forceful, that the small defect caused by the operation will heal over in a very short time, thus restoring the original state of affairs.

A permanent occlusion, however, would be assured, if instead of closing the linear incision in the seromuscularis, the stomach were to be divided and the two stumps buried in the pyloric and duodenal end respectively.

This modified method of Biondi is performed in the following manner: Gastroenterostomy. The gastrohepatic ligament is ligated in the pyloric region, which enables us to deliver the pylorus in front of the abdominal wall. The transverse incision through the seromuscularis (Fig. 2) is then carried around the pylorus. The muscularis is peeled away from the mucosa which is thus exposed intact for about one inch. The mucosal tube is ligated above and below (Fig. 3) with a silk or Pagenstecher ligature, cut in between the ligatures (Fig. 4), and the stumps carbolized. Figure 5 shows the burying of the stumps on the stomach end and on the duodenal end.

The experimental work was done along two

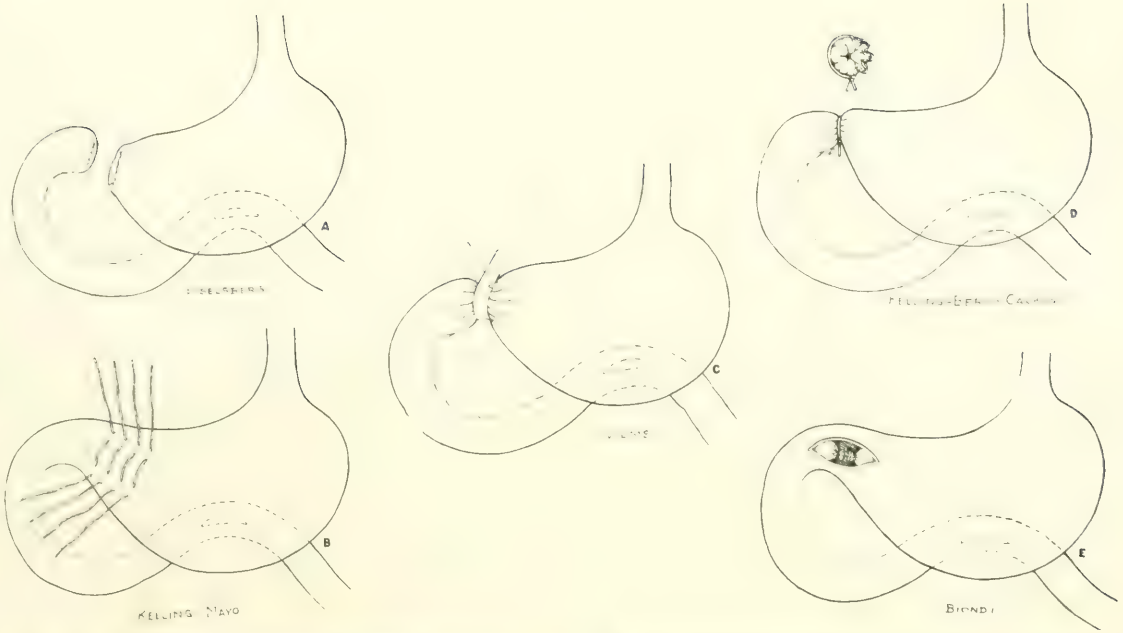

Fig. 1*A*. Von Eiselsberg method of pyloric exclusion.
Fig. 1*B*. Kelling-Mayo method of pyloric exclusion.
Fig. 1*C*. Wilms method of pyloric exclusion.

Fig. 1*D*. Kelling-Berg-Cackovic method of pyloric exclusion.
Fig. 1*E*. Biondi method of pyloric exclusion.

lines: (1) Exclusion of the pylorus according to Biondi, and (2) operations with the modified method, just described.

1. *Exclusion of the pylorus Biondi*. This method was applied on five dogs. One dog, which showed some signs of distemper on the day of operation, died the next day. The other four dogs were observed a sufficiently long time to make the post-mortem findings of value. The results are the following: The occlusion was in place after fourteen days, (dog 256). After twenty-one and thirty-four days (dogs 320 and 96) the lumen had become re-established and admitted a medium-sized glass rod. The findings after eighty-seven days are shown in Fig. 6 (frontispiece). The only trace of any operative interference is one Pagenstecher stitch attached to

Fig. 2. Modified Biondi method showing incision of serosa and muscularis.

the mucosa and protruding into the lumen of the stomach; otherwise the field of the operation in the pyloric region appears to be perfectly normal.

Microscopical examination (Fig. 7) (Pathological Accession No. 3,531): This section shows a portion of the pyloric end of the stomach and adjacent part of the duodenum. The mucous membrane is intact throughout (except for some artefacts in the section) and appears normal. The muscularis mucosæ is intact too throughout the length of the specimen. The submucosa appears somewhat thicker than normal and is composed of very dense fibrous tissue. Except for the thickening of the submucosa the section resembles a normal section of this portion of the gastro-intestinal tract.

2. *Exclusion of the pylorus (modified Biondi).* This series comprises five cases. In one case (dog 116) the result was spoiled by an error in technique. In peeling away the muscularis from the mucosa the latter was accidentally opened at the duodenal side and the intestinal contents escaped. The dog died the following day and the post-mortem examination showed a leakage of the duodenal stump. Another dog (29) died four days after the operation from an unknown cause. The operative result was perfect. Three dogs (103, 244, 155) were observed thirty-eight, sixty, and one hundred and fifty-four days respectively. In all of them the exclusion was perfect. This modification insures a permanent and absolute occlusion of the pylorus.

The advantages of the original Biondi method and its modification, as compared

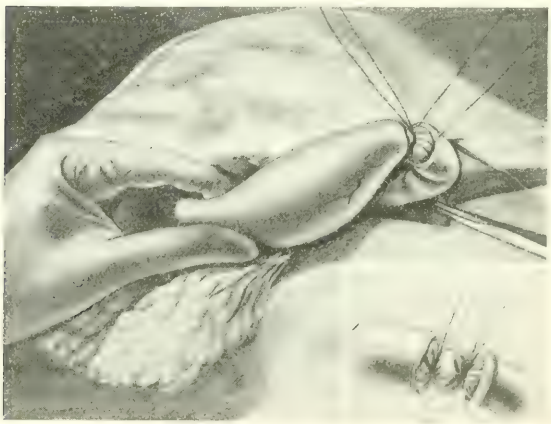

Fig. 3. Modified Biondi method showing ligation of mucous membrane.

with the Eiselsberg method are the following: No extensive ligation is required. No clamps which take up a great deal of space are needed. The Biondi method does not form large stumps and therefore makes the infolding of these stumps much easier than does Eiselsberg's method.

The Biondi method would therefore be preferable to the Eiselsberg method, if the technique were an absolutely safe one. The technique, however, is by no means as simple, as Porta has claimed. The dissection of the

muscularis from the mucosa is not as easy as appears from his description. My records show that during this process the mucosa was accidentally injured a few times. The great risk of this accident is obvious. As no clamps are applied in the Biondi method, leakage of intestinal contents and infection of the peritoneum are grave dangers of this procedure. Gibson and Beekman have already drawn attention to this difficulty in Biondi's technique. For the same reason, Strauss' method, a combination of the method of Wilms and Biondi, cannot be considered absolutely safe, though the results of his animal experiments are certainly very satisfactory.

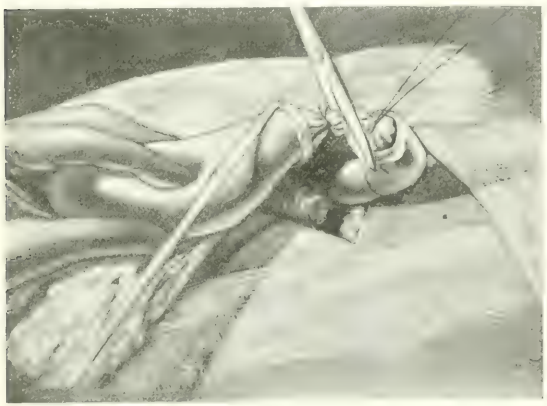

Fig. 4. Modified Biondi method showing division of mucous membrane.

This technical difficulty, however, would not prejudice us against this method, if it were really to assure a permanent occlusion of the pylorus. The specimen represented in Fig. 6 (frontispiece) shows conclusively, however, that the original Biondi method does not guarantee a permanent occlusion. It is really remarkable to observe the regenerative power of the gastro-intestinal mucosa of this specimen. Ligation of the mucous membrane in two places, division of the same between these ligatures, and carbolization of the stumps did not leave after two months a

trace of any surgical interference, except one stitch still *in situ*.

If the Biondi method, as shown from our experiments, does not safeguard against reopening of the pylorus, we certainly cannot expect any permanent occlusion from the infolding and constriction methods (Mayo, Kelling, Berg, Wilms, etc.). We must agree with Leriche, who came to the conclusion that with the exception of Eiselsberg's method all the different forms of pyloric exclusion are "un parodie du l'exclusion vraie." It is perfectly true that none of these methods guarantee a permanent occlusion of the pylorus.

As far as the permanent result is concerned

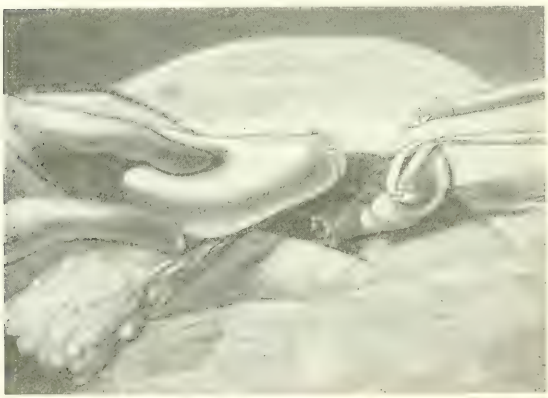

Fig. 5. Modified Biondi method showing the burying of the stumps.

we cannot therefore agree with the statement of Berg that his method "effectually and permanently excludes the antral end of the stomach and duodenum from the passage of stomach contents through them."

That this statement is correct is proved by a human pylorus which I had occasion to resect a few months ago. The rarity of this specimen induces me to give the history somewhat in detail.

B. C., aged 24 years, was operated on by Dr. A. A. Berg, at the Beth David Hospital, for duodenal ulcer, in September, 1913. A gastro-enterostomy

was made and the pylorus was occluded by an encircling stitch. The patient was relieved from her symptoms for a few months only. Afterward her symptoms reappeared and she entered the Montefiore Home. An exploratory laparotomy was performed by Dr. Charles Goodman, May, 1914. He found the gastro-enterostomy wide open and the pylorus patent. The upper part of the small intestines was studded with small grayish nodules. Diagnosis; Tuberculous peritonitis. After the second operation she was relieved of her symptoms

Fig. 7. Microscopical section of pyloric region 87 days after Biondi operation, Dog 240, No. 3,581. Path. Surg. (×30).

for a while and then her old symptoms recurred. Radiography showed retention of food in the pyloric pouch, distal to the gastro-enterostomy. Though the possibility of a gastric neurosis (hysteria) was considered, it was deemed possible that a resection of the stomach near the gastro-enterostomy might relieve her symptoms.

Operation, July, 1915 (Lewisohn): Gas and ether anæsthesia: right pararectal incision; gastro-enter-

ostomy is patent. The large intestine is of normal appearance, not dilated, no nodules on its surface. The small intestine is of normal caliber. The walls are soft, no ulcers are felt. On the surface of the proximal part of the ileum a few whitish nodules are noticeable. The pylorus is patent, admitting the little finger (Fig. 9). No ulcers are palpable at the pylorus or in the duodenum. On the anterior surface of the pylorus the old Pagenstecher stitch, situated subperitoneally, is still in evidence. Resection of the pylorus and layer suture of both ends.

The patient was again free from vomiting for a while. Her symptoms, however, reappeared. Evidently she is suffering from a neurosis.

The specimen of the pylorus shows the stitch on the anterior surface, slightly curved; the knot is distinctly visible and intact. The mucous membrane does not seem to be atrophic on microscopical appearance. No remains of the stitch are noticeable on the posterior surface of the pylorus.

Microscopical examination of section from the anterior wall (Accession No. 3664) (Figs. 10 and 11):

Fig. 9. View of pyloric lumen 20 months after pyloric exclusion (patient B. C.).

The wall of the pylorus (Fig. 10) appears considerably thickened at the constricted portion. The mucous membrane appears normal except for slight thinning of the constricted portion. The submucosa is much thickened, composed of dense connective tissue. The remains of the suture can be seen surrounded by an area of thickened connective tissue, round cells and giant cells. The muscularis is thickened at the point of constriction, the thickening being due to the increase in the connective tissue. Figure 11 shows very well finer details of the fate of the threads, distinctly surrounded by a ring of connective tissue; a great mass of inflammatory tissue and numerous giant cells are distinctly seen in this section. A bridge of connective tissue has grown between the two parts of the double thread, separating them entirely.

Microscopical examination of section from the posterior wall (Accession No. 3690) (Figs. 12 and 13): A section from the posterior wall (Fig. 12)

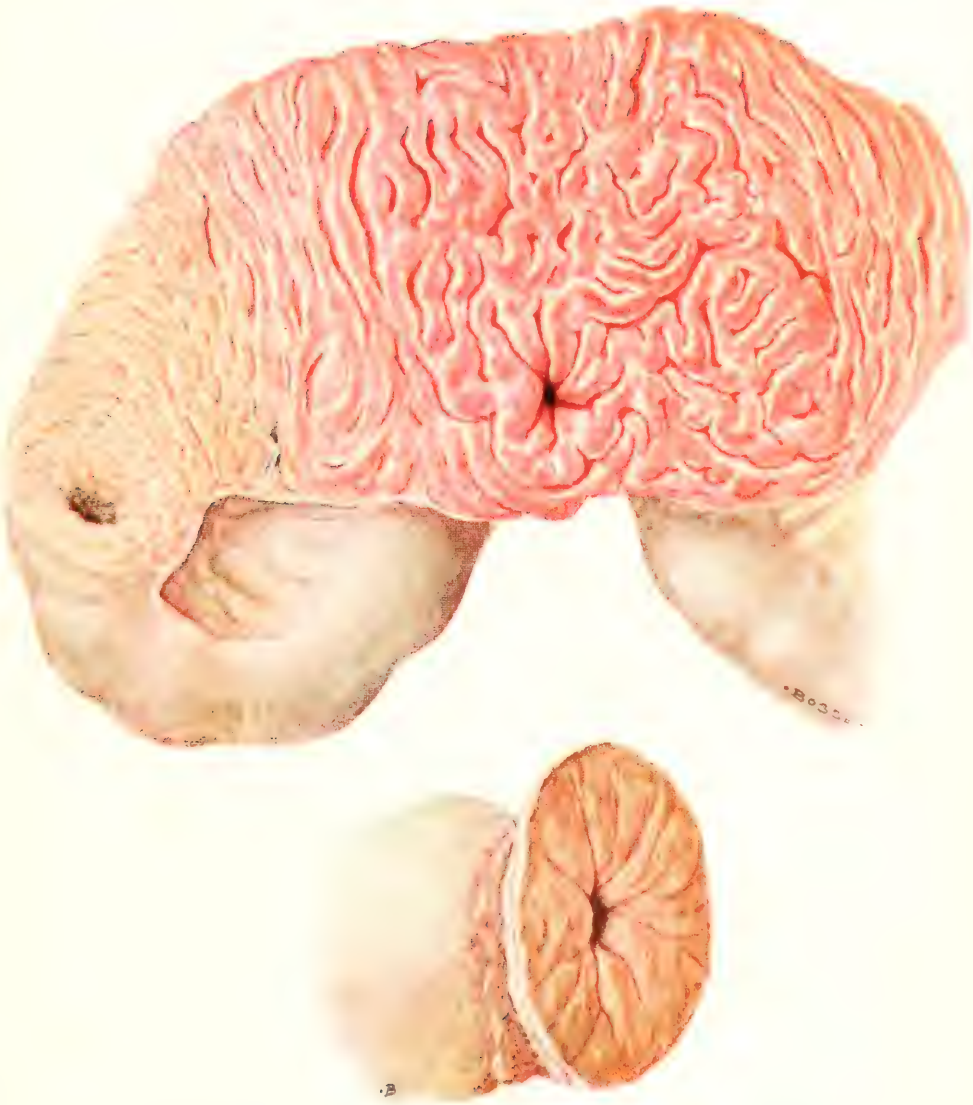

Fig. 6 (above). Stomach of Dog 246, 87 days after Biondi operation.
Fig. 8. Resected pylorus (patient B. C.). Stitch still in place, 20 months after pyloric exclusion. Pylorus patent.

(*Richard Lewisohn.*)

shows the same thickening of the submucosa, but in a much less marked degree. At the point of constriction the serous coat is very much thickened by an increased amount of fibrous tissue, in which the remains of the suture are visible. The muscularis is interrupted at this point by a narrow band of fibrous tissue, which runs from the submucosa to the serous coat. The mucous membrane appears entirely normal. Figure 13 shows finer details of the stitch and surrounding tissues. The findings are

Fig. 10. Microscopical section of anterior wall of pylorus (patient B. C., Surg. Path. No. 8,6041). a Exclusion stitch (×30).

about the same as in Fig. 11. The mass of inflammatory tissue is larger than in the corresponding section from the anterior wall. The giant cells are not quite as numerous.

This specimen shows conclusively that the occlusion was not a permanent one. This is in accordance with radiographic findings re-

ported from different clinics. Radiographs taken a year or more after the exclusion show a re-opening of the pylorus in a great majority of cases. Pictures taken a few months after the exclusion are of no value; for it can be well conceded, that all these methods provide for a temporary exclusion of the pylorus. The re-establishment of the pyloric lumen following occlusion may be explained by the following hypothesis:

Fig. 11. Same as Fig. 10. Threads surrounded by a dense ring of connective tissue, round and giant cells. (×200).

The constricting stitch, placed to occlude the pylorus, by its pressure gradually cuts through a part or the whole of the peritoneum, similarly through muscularis, through submucosa and mucosa. This process continues until the stitch has cut to the point where pressure is no longer exerted upon the tissues.

Repair of the divided structure proceeds immediately behind the ligature, as it advances through the tissues. Thus at no time is there an opening in the intestinal wall. When the ligature comes to rest it is sometimes free in the pyloric lumen, sometimes it is embedded in the wall of the pylorus, as any non-absorbable suture material may be. The actual cutting process caused by the stitch takes place through degeneration, death and dis

Fig. 12. Microscopical section (Surg. Path. No. 3,690) of posterior wall of pylorus (patient B. C.). *a* Exclusion stitch. (×30).

integration of the cells. The local compression of the vessels and cells deprives the tissue of nutrition, causing cell degeneration and death. Autolytic ferments and phagocytic activity lead to the cell disintegration and removal. The connective-tissue stroma, as the tissues come together behind the suture,

proliferates, repairing the defect from the loss of cells. Cicatricial tissue therefore repairs and leads to regeneration of the intestinal wall.

I think that the arguments about the permanent anatomical exclusion of the pylorus and about the preference of one method over the other are rather futile. We can grant that with the exception of Eiselsberg's method or the modified Biondi method above described,

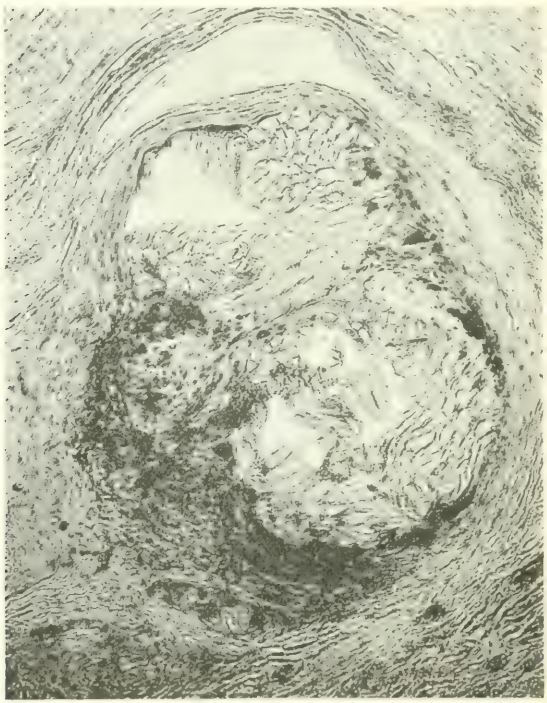

Fig. 13. Same as Fig. 12. Same findings as in Fig. 11. The defects in the cross section of the linen threads are artefacts, caused by the microtome knife. (×200).

none of the exclusion methods will insure a permanent occlusion. The argument is really academic only. The patient consents to an operation, because he wants to be relieved of his symptoms. It is immaterial to him, whether his pylorus is patent after a certain time, as long as he is permanently cured.

It is evident that all we have to do to obtain that result is to prevent food and stomach juice from coming in contact with the ulcer for a few months (or possibly for a few weeks only). A temporary absolute exclusion of the pylorus, not a permanent one, is needed. This purpose is just as well obtained by the exclusion stitch with a Pagenstecher suture, which seems to be technically the most simple procedure, as by any of the other more complicated methods (Wilms, Biondi, etc.).

CONCLUSIONS

1. With the exception of Eiselsberg's unilateral exclusion and the modified Biondi method, none of the different methods of exclusion guarantees a permanent, occlusion of the pylorus.

2. An absolute, though temporary exclusion of the pylorus provides for a permanent cure of pyloric and duodenal ulcers.

3. The most simple method from a technical standpoint is the exclusion stitch (Kelling-Berg-Cackovic). This stitch should be used in preference to the more complicated methods (Wilms, Parlavecchio, Biondi).

4. The Eiselsberg's method and the modification of the Biondi method, though guaranteeing a permanent exclusion, are technically too complicated and should not be used.

5. The clinical results are just as good in using the most simple method (exclusion stitch) as in the use of the most complicated method (Eiselsberg). The exclusion stitch is therefore the method of choice for the treatment of pyloric and duodenal ulcers.

RECORDS OF ANIMAL EXPERIMENTS

A. BIONDI METHOD

DOG 348. Previous gastro-enterostomy had been done by students, May 3, 1915. Dog has some distemper. *Operation*, May 11, 1915: Pararectal incision, pylorus brought forward, pyloric artery tied; seromuscular coat incised and liberated from mucosa; mucosa ligated above and below (silk) and cut in between; stump carbolized and buried; layer suture of abdomen. May 12: Dog died of distemper. *Post-mortem examination:* No peritonitis;

gastro-enterostomy patent. Exclusion stitches in place. *Observation time*, one day.

Dog 256, Surg. Path. No. 3,407. *Operation*, March 23, 1915: Gastro-enterostomy posterior antecolica; gastrohepatic ligament ligated and cut, thus facilitating protrusion of pylorus in front of abdominal wall; pylorus cut, mucous membrane ligated in typical fashion; central ligature, cut by mistake, reapplied; seromuscularis sutured; layer suture of abdomen. April 6: Killed on account of distemper. *Post-mortem examination:* Peritoneum smooth; gastro-enterostomy functionating; exclusion evidently holding. *Observation time*, fourteen days.

Dog 320, Surg. Path. No. 3,485. *Operation*, April 27, 1915: Median laparotomy; suture gastro-enterostomy; longitudinal incision of seromuscularis over pyloric region; mucosa freed all around and cut through between two ligatures; stumps covered by musculoserosa suture; abdomen closed. May 17: Died of bronchopneumonia. *Post-mortem examination:* Gastro-enterostomy patent. Pylorus is patent again for medium-sized glass rod. *Observation time*, twenty-one days.

Dog 96, Surg. Path. No. 3,251. *Operation*, November 24, 1915: Typical gastro-enterostomy posterior antecolica; exclusion of pylorus; small longitudinal incision over pyloric region through serosa and muscularis; attempt to strip muscularis from mucosa very difficult as tissues are adherent to one another; therefore, transverse incision added, encircling most of the stomach. December 28: Died of lobar pneumonia. *Post-mortem examination:* No peritonitis. Specimen removed *in toto*. Pylorus patent. *Observation time*, thirty-four days.

Dog 246, Surg. Path. No. 3,531. *Operation*, March 17, 1915: Gastro-enterostomy posterior antecolica; typical pyloric exclusion according to Biondi. Exclusion technique worked very well in this case. Stumps covered with seromuscularis coat. June 8: Killed. *Post-mortem examination:* Removal of stomach, duodenum, and upper part of jejunum. Gastro-enterostomy patent. No outside scar visible in pyloric region. Omentum slightly adherent to this region. The pylorus is perfectly open, exactly like a normal pylorus. Stomach opened along the lesser curvature, into the duodenum. The pyloric-duodenal junction is perfectly normal. One Pagenstecher stitch lying in this region is the only sign of operative interference (Fig. 6 frontispiece). *Observation time*, eighty-seven days.

B. MODIFIED BIONDI METHOD

Dog 116. *Operation*, December 8, 1914: Gastro-enterostomy posterior antecolica with clamp; pylorus cut on its anterior surface through serosa and muscularis, after freeing the pylorus from its

attachment (gastrohepatic ligament). In trying to pass an aneurism needle around the mucous membrane, the latter was pierced and some stomach contents escaped. Suture of both duodenal and stomach end of the mucosa, inverting suture of both stumps, which is very unsatisfactory on the duodenal end; closure of abdominal wall. December 9: Died of peritonitis. *Post-mortem examination:* Peritonitis in upper abdomen; leakage of duodenal stump. Stomach end of resection in good condition and gastroenterostomy perfect. *Observation time,* one day.

Dog 129. *Operation,* December 15, 1914: Typical gastrojejunostomy; pylorus freed all around and brought outside of abdominal parietes; transverse section through serosa and muscularis in anterior and posterior aspect of stomach; ligature of mucous membrane above and below (Biondi), and inversion of stumps of stomach and duodenal end, closure of abdominal wall in layer sutures. December 19: Died. *Post-mortem examination:* No peritonitis; exclusion in good condition; specimen lost in transit. *Observation time,* four days.

Dog 103, Surg. Path. No. 3,261. *Operation,* December 1, 1914: Typical gastro-enterostomy posterior antecolica; a cross incision made over the pylorus on the anterior wall of the stomach, and serosa muscularis peeled back from mucosa; an aneurism needle was carried around the mucous membrane at the posterior wall and the mucous membrane ligated between two silk ligatures and cut in between; the stumps were carbolized; the rest of the seromuscularis coat was now divided and a running suture united the incision at the stomach end, thus burying the mucous membrane stump. Same procedure for duodenal end. Suture of abdominal wall. January 7, 1915: Dog died. *Post-mortem examination:* Bronchopneumonia. Peritoneal cavity normal. Exclusion perfect. No local peritonitis around excluded part. Right middle lobe of lung shows extensive pneumonia. *Observation time,* thirty-eight days.

Dog 244, Surg. Path. No. 3,544. *Operation,* March 16, 1915: Gastro-enterostomy posterior antecolica; longitudinal incision one inch long across pylorus, cutting through serosa and muscularis; the stripping off of the muscularis from the mucosa successful only after small transverse incision had been added; pin-hole opening accidentally made in mucosa; double silk ligature then carried around on aneurism needle, tied proximally and distally and mucosa cut through in between; stumps carbolized; musculoserosa incision sutured over stumps. June 15: Killed. *Post-mortem examination:* Gastro-enterostomy patent. Pyloric exclusion perfect at both ends (stomach and duodenum). *Observation time,* sixty days.

Dog 155, Surg. Path. No. 3,543. January 12, 1915: Typical posterior gastro-enterostomy. Pylorus then cut across transversely after ligating gastrohepatic ligament. Cut down to mucosa. Muscularis stripped away from mucosa with some difficulty. Ligatures tied around mucosa sac above and below and cut in between. As both ligatures were too near each other, they slipped off. Openings closed with suture and then buried. Layer suture of abdomen. June 15: Killed. *Post-mortem examination:* Gastro-enterostomy patent. Inner silk suture still in place on one spot. Occlusion of pyloric end of stomach and duodenum perfect. *Observation time*, one hundred and fifty-four days.

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THE PROCESS OF REPAIR IN WOUNDS OF THE SMALL INTESTINE

By JOHN E. McWHORTER, M.D., A. P. STOUT, M.D., and CHARLES C. LIEB, M.D., New York

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THE PROCESS OF REPAIR IN WOUNDS OF THE SMALL INTESTINE[1]

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IN accordance with a general scheme suggested by the Director of the Surgical Laboratory, to study the phases of repair in the body systems, we have selected the problem of repair in the gastro-intestinal tract with the hope that data might be obtained that would be of practical value in the post-operative care of intestinal suture. The small intestine was chosen first because the undergraduate course in operative technique afforded abundant material. Later we hope to supplement this work with similar observations on the large intestine and stomach.

The objects of these experiments are: *first*, the determination of the hydrostatic pressure that a repairing intestinal wound will withstand; *second*, the effect of transverse division and suture on segmentation; and *third*, an observation of the gross and microscopic phenomena of repair.

HISTORY

In 1887 Senn (1) described experiments on the production of obstruction, volvulus, strangulation, and intussusception, together with the results obtained from resection and subsequent suture of the intestine. During the course of his experiments he reported, though not in detail, a few of the processes of repair.

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In one series of experiments he produced obstruction by tying the intestine near the ileocæcal valve. Three to seven days later he performed an ileo-ileostomy or ileocolostomy, using decalcified bone plates. One of his dogs died at the end of 24 hours. When the intestine was subjected to hydrostatic pressure, no leakage occurred at the site of suture. He believed that absolute physiological rest of the intestine was necessary for perfect healing. He also reported that scarification or chemical irritation of the peritoneum produced adhesions that were both firmer and more rapid in their development than those following simple apposition of serous surfaces by suture. After mechanical or chemical irritation of the peritoneum an outflow of blood and "lymph" appeared on the surface. He also noted that gross vascularization began after 40 hours. At 15 days the mucosa was almost but not quite completely regenerated.

In 1896 Mall (2) presented a detailed picture of intestinal repair. He noted that within a few hours after the suture, the serous surfaces were adherent and he believed that fibrinous union took place before the operation was completed. At the end of 24 hours the inverted cut edges of the intestine showed extensive coagulation necrosis. After 48 hours, in the sulci between dead and living mucosa, epithelial cells of embryonic type were proliferating in all directions. At the end of 6 days there was a downgrowth of glands into the submucosa, probably because the needle tore through the bottom of the crypts. Many of these glands were cystic. At the end of 14 days, the approximated layers of the muscular and submucous coats were united by firm fibrous tissue. The denuded surfaces of the mucosa were covered with granulation tissue until the crypts and villi had regenerated. On the fifteenth day the mucosa was almost completely regenerated. After 24 days regeneration was complete. When the mucosa had healed the inverted

parts unfolded and the intestinal wall straightened out into a smooth, unbroken line. At the end of two months, all the coats were fully regenerated.

He divided the processes of healing into four stages: *first*, the immediate fibrinous union of the serous surfaces; *second*, the destruction of the parts protruding between the flaps of mucosa; *third*, the regeneration of the mucous membrane; *fourth*, the straightening out of the suture line.

Gould (3) confirmed most of Mall's findings but noted in addition that the plastic exudate uniting the serous surfaces, extended from 3 to 5 cm. beyond the line of suture. At the end of three days some 3 to 5 mm. of fatally injured mucosa had sloughed and the submucosa was œdematous. Many new connective-tissue cells appeared in the clot between the approximated serous surfaces and complete organization took place at the end of the eighth day. In one case he found that the denuded surface was covered with embryonal epithelium at the end of eight days. After 21 days the denuded area was completely covered by the newly formed mucosa but the muscularis mucosæ had not developed. The muscularis was approximated by the contraction of the intervening scar-tissue.

According to von Frey (4) a plastic exudate glued together the approximated serous surfaces of the sutured intestine. He made no direct statement relative to muscular regeneration although in his drawings of sections taken two days to four months after operation, the muscularis had not regenerated.

Hoffman (5) showed that small defects in the mucous membrane of the stomach and intestine of dogs healed completely. A defect of 5 by 2 cm. had regenerated completely at the end of 32 days, excepting for a small area about 4 mm. in diameter. Larger defects were covered with a single layer of epithelium, while very large defects were covered with granulation tissue.

Meek (6) reported that the longitudinal

muscular coats of the cat's intestine regenerated in from 7 to 9 days after division and suture.

Cannon (7) in a discussion of the relative merits of end-to-end and lateral anastomosis, gives his results from observations made on different animals, one, four, seven, and ten days after end-to-end union of the intestine. He found that "in no case was the slightest evidence observed of stasis of the food in the region of the operation." In lateral anastomoses, however, he found at the end of ten days to two weeks, a more or less complete blocking of the canal by accumulated hair and undigested detritus at the opening between the apposed loops.

TECHNIQUE OF OPERATIONS

The experiments were divided into two series; one upon the relatively normal small intestine and the other upon an intestine that had been previously injured.

Series A. The animals in this series were dogs of various types, unselected and often poorly nourished. The operations were done by fourth year students, who had had little if any previous operative experience. Each operation was supervised by an instructor, who, however, did no part of the operation. There were three varieties of operations on the small intestine; the repair of enterotomies, lateral anastomoses, and end-to-end anastomoses. The method of suture varied somewhat, but as a rule an inner layer of through-and-through continuous or lock stitch was taken, and this was infolded by some form of continuous seromuscular suture. The suture material was invariably white silk. Since the course is particularly designed to teach operating-room asepsis, special care was taken to maintain as perfect a technique as possible. All the operations of exposing the lumen of the gut were done with a change of towels and instruments between the middle contaminated and the terminal clean stages.

The care of the dogs was as follows: The

day before the operation the dogs were given a soap and water bath and the field of operation was cleansed of hair with barium sulphide mixture. All the operations were done in the forenoon, and on that day the dogs received no food at all. The anæsthetic used in every case was ether. Following all operations the dogs were given, as a routine, water on the day of operation, milk and water the next day, and regular diet of bread, meat, and small bones on all succeeding days.

Series B. After completing the first series, the objection was raised that the experiments were inconclusive, since the operations were done on the normal intestine. Consequently we endeavored to injure the gut by interfering with its blood supply before doing the suture operation. Some difficulty was encountered before a satisfactory method was found. Ligation of the mesentery which included the vessels supplying a loop of intestine a foot in length, had apparently no effect on the viability of the gut. Injecting liquid paraffin into a branch of the mesenteric artery or vein was a little more successful but could not be controlled. Sometimes a loop became moderately hyperæmic but more often there was no effect at all, or there was a general mesenteric thrombosis with gangrene and quick death. Finally a simple, effective and easily controlled method was devised: a piece of half-inch tape was tied about a loop of intestine just tight enough to cut off the venous return while still allowing the entrance of arterial blood. This simulates exactly strangulation of the gut as seen in strangulated hernia, volvulus, and like conditions. This preliminary operation was done twenty-four hours before the second operation, which consisted of wide resection of the strangulated gut and end-to-end anastomosis. In this second series the preliminary operation was done by the experimenters and the resections and anastomoses were done in some cases by students, in others by the experimenters

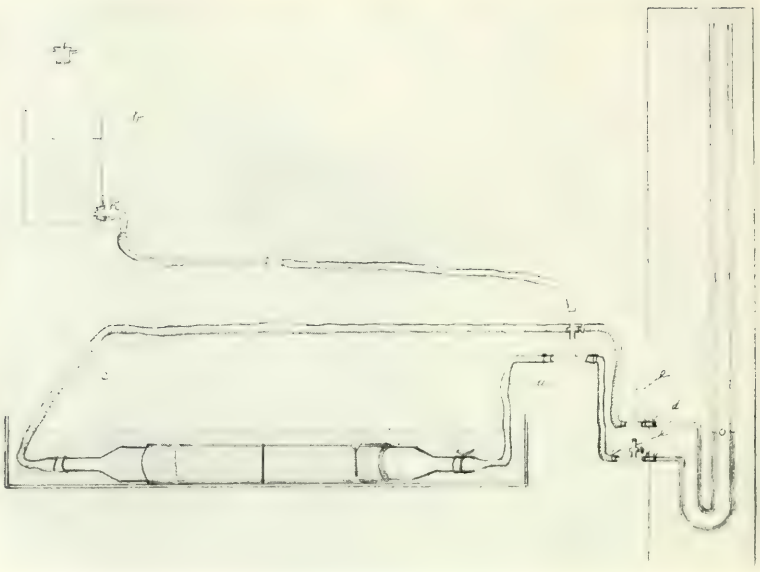

Fig. 1. Diagram of water manometer.

themselves. The method of anastomoses was that already described.

HYDROSTATIC EXPERIMENTS

Technique of experiments. The specimens of both series were handled in exactly the same way. The dog was killed or died at periods from 25 minutes to 144 days following the suture operations. The abdominal cavity was opened as soon as possible after death and a loop of gut was excised which included the area of operation and six inches or more on each side of it. This segment was then treated in either one of two ways. Some had one end tied off and a glass syringe fastened into the lumen at the other. Water was then introduced until the loop was considerably distended. During the distention the suture line was under observation for leakage.

The other specimens were subjected to measured pressure as follows: After removal from the body, the specimen was immersed in warm Ringer's solution and glass cannulæ

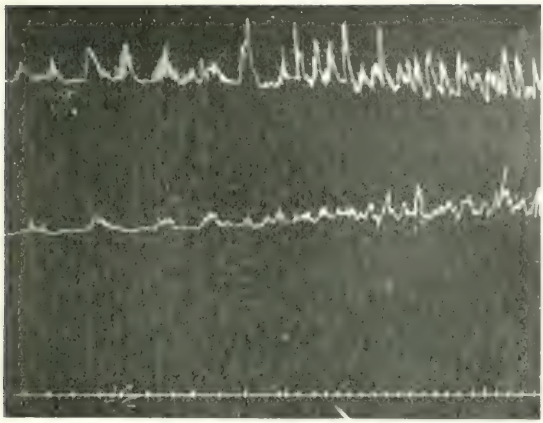

Fig. 1A. Movement of circular coat of intestines one hour after end-to-end anastomosis. Upper tracing made by circular coat 95 mm. caudal to anastomosis. Lower tracing made by circular coat 15 mm. cephalaid to suture. During contraction of the muscles lever moves upward. Lowest tracing time in minutes. Note change in speed of kymograph.

were inserted into the lumen at either end and securely tied in place. While the gut was still submerged, both ends were connected to the apparatus by means of rubber tubing. One of the connected ends (Fig. 1, *a*) led to a water bottle (Fig. 1, *b*) containing colored Ringer's solution. This bottle was hung from the ceiling and by means of a rope and pulley could be raised and lowered to any desired height, thus giving various hydrostatic pressures. The rubber tubing at the other end (Fig. 1, *c*) was attached to the short arm of the water manometer (Fig. 1, *d*). With the intestine connected to the apparatus, the air was expelled from the lumen of the intestine as well as from the various lengths of tubing, by raising the water bottle. A by-pass (Fig. 1, *e*) with stop-cock in front of the manometer allowed for the escape of air without the disturbance of fluid level in the manometer tubes. Next the water bottle was brought to the level of the intestine and the stop-cock was opened. The level of the fluid in the long arm (Fig. 1, *o*) of the manometer was noted and marked zero, particular care

being taken to see that this point did not represent either negative or positive pressure. To obtain positive pressure within the lumen, the water bottle was gradually raised to any desired height, and the pressures were calculated by measuring the height to which the fluid rose in the long arm. Any leakage was at once detected, for the Ringer's solution in the water bottle was colored blue with *acridin blau*, a vital stain.

Results of the hydrostatic experiments. As may be seen by referring to Tables I and II, thirty-two specimens in Series A and B, were subjected to various degrees of hydrostatic pressure from within the lumen. As the results in the two series were the same, they will be considered together. Six leaked. Of the twenty-six that showed no leakage, nineteen followed suture of supposedly normal gut, while seven followed resection of gut showing more or less severe vascular changes. The experiments were done at from twenty-five minutes to 144 days following the suture operations. The measured pressures which the specimens withstood without leakage varied from 350 to 1400 mm. of water (one-half to two pounds per square inch). That these pressures far exceed any that under ordinary circumstances exist within the living intestinal lumen, may be readily appreciated by considering for a moment the normally functionating intestine.

The actual amount of positive pressure in the intestinal lumen *in vivo* is unknown. The pressure varies considerably even under normal conditions, for, during the intervals of digestion when the intestine is quiescent, the pressure is practically zero. During digestion, however, with the passage of the bolus along the canal, the internal pressure at any one time and upon any given area is certainly positive, but the actual amount is relatively slight. Even under abnormal conditions of distention, although the pressure is undoubtedly far greater than the average, it unquestionably never approaches anywhere

near the amount to which the above tabulated specimens have been subjected.

Wyllis Andrews (8), working with ox intestine and human intestine freshly removed from the body, found that they burst at an internal air pressure of from 6 to 10 pounds per square inch. Senn (8) experimenting on the cadaver in a case of enteritis, found that the diseased bowel ruptured at an air pressure of 1½ pounds per square inch.

Muller (9) in a recent article on the bursting strength of the alimentary tract, found that in cats the average pressure necessary to rupture the jejunum was 28.2 pounds per square inch (1458 mm. Hg.), and the ileum was 24.8 pounds per square inch (1254 mm. Hg.). This pressure varied considerably in different cats but was uniform in the same individual. He further noted that the tear was linear and ran in the long axis of the organ and that the strength of the alimentary tract lay chiefly in the submucosa.

The six cases of leakage above recorded, together with a number of similar cases examined but not included in the tables were, with the exception of one case, invariably associated with local infection about the suture line or a general peritoneal infection. The one exception was that of a lateral anastomosis done by one of the students (Table I, No. 1) and tested twenty-five minutes after completion of the suture operation, and fifteen minutes after the death of the dog from the anæsthetic. The leakage here was due undoubtedly to faulty operative technique. What the source of the infection was in the other cases could not be definitely ascertained. In many, a visible hole leading into the lumen was present, and in these cases the leak probably preceded and caused the infection. That other lesions without infection can be present in the intestinal tract without interfering with the repair, is seen from cases seven and fourteen of Series A (Table I). These dogs died respectively of mesenteric thrombosis and intussusception,

and yet showed a resistant suture line. That very slight local infection is of no consequence is amply proved by the relatively enormous pressure that some of these specimens withstood though the microscopic picture was that of infection. Given, however, a marked infection whether accompanied by gross suppuration or not, leakage will take place at pressures so slight that they cannot be recorded by the manometer.

In connection with this work on the living intestine, it is of interest to note the two following experiments. A dog was chloroformed and at death the small intestine was divided in two places and immediately joined by end-to-end anastomosis. One-half hour later, one of these sections was removed and subjected to pressure of 850 mm. of water without leakage. Two hours later the second section was excised and subjected to a pressure of 1250 mm. without evidence of leakage.

VIABILITY OF THE INTESTINE

It has been shown in a previous section that an intestine can withstand, within one hour after an anastomosis, an internal pressure varying between 330 mm. and 1400 mm. of water. It seemed worth while to determine whether such operations as anastomoses interfered with the viability of the gut.

No better index of the viability can be offered than a graphic record of the movements of a loop of intestine which includes an anastomotic suture. A preliminary experiment with a loop of dog's intestine one hour after the repair of an enterotomy showed that this operation did not interfere with the contractions of the longitudinal muscular coat. Such an experiment would not indicate whether the contractions were due to movements of muscle-fibers in close proximity to the injury or to those fibers at a considerable distance from the site of operation.

In order to determine the relative activity of smooth muscle near the site of operation and at a considerable distance therefrom, the following experiments were devised:

Immediately after the suture had been tested for its efficiency against internal pressure, the cannulæ which had been tied into the lumen were removed and the loop of intestine was transferred to an oblong dish containing fresh, warm Ringer's solution. The loop of intestine was then fixed by means of two sutures to a glass tube, the ends of which were bent at right angles. Exactly opposite the two original sutures, two other stitches were taken and tied. One end of each of these threads was then passed over a pulley and connected to a recording lever which wrote upon the smoked paper of a slowly-moving kymograph. The levers were properly weighted, and wrote in such a fashion that when the circular muscle of the intestinal loop contracted the writing point moved upward; during the relaxation of the intestinal wall, the writing point descended. Oxygen was supplied to the Ringer's solution which bathed the intestinal loop, and the temperature of this solution was maintained at 38° to 39° C. by means of a large water-bath.

The position of the sutures varied in the different experiments, so that we were able to study muscle within 5 mm. of the site of operation as well as muscle 100 mm. from the line of suture. The accompanying illustration (Fig. 1A) is a graphic record made by the circular muscle of a loop of intestine which was excised one hour after an end-to-end anastomoses had been made. The lower tracing is made by the circular coat 15 mm. cephalad to the suture; the upper tracing represents the movements of the circular coat 95 mm. caudal to the anastomosis. The movements of the muscle 95 mm. from the site of injury are powerful, regular, and show very distinct tonus waves. The movements 15 mm. from the injury are fairly regular and show less pronounced variations in tonus.

Though it is true that all the movements that we have recorded are comparatively small, this may be accounted for, first, by the fact that these loops have invariably been

TABLE I

Exp. No.	Surg. Path. No.	Time Post-op.	Type of Operation	Cause of Death	Leak or No	Pressure How Obtained	Mm. of Water Press	Lbs. per Square Inch
1	3297	25 minutes	Enterorrhaphy	Died on table, anæsthesia	Slight	Syringe		
2	3250	1 hour	Enterorrhaphy	Killed, ether	No	Water manometer	350	
3	3202D	1 hour	Lateral anastomosis	Killed, chloroform	No	Syringe		
4	3273	1 hour	End-to-end anastomosis	Killed, ether	No	Water manometer	170	
5	3280A	24 hours	Enterorrhaphy	Killed, chloroform	No	Syringe		
6	3312	24 hours	End-to-end anastomosis	Killed, chloroform	No	Water manometer	850	
7	3323	24 hours	Enterorrhaphy	Died mesenteric thrombosis	No	Syringe		
8	3324	24 hours	Enterorrhaphy	Died general peritonitis	Leak	Syringe	0	0
9	3310	48 hours	End-to-end anastomosis	Killed, chloroform	No	Water manometer	820	
10	3275	48 hours	End-to-end anastomosis	Killed, chloroform	No	Water manometer	870	
11	3335	3 days	End-to-end anastomosis	Died general peritonitis	Leak	Syringe	0	0
12	3344	4 days	End-to-end anastomosis	Killed, chloroform	No	Water manometer	860	
13	3282	6 days	End-to-end anastomosis	Killed, chloroform	No	Syringe		
14	3329	14 days	Enterorrhaphy	Died, intussusception	No	Syringe		
14	3329	14 days	Enterorrhaphy	Died, intussusception	No	Syringe		
15	3204	31 days	End-to-end anastomosis	Killed, chloroform	No	Syringe		
16	3203	38 days	Lateral anastomosis	Killed, chloroform	No	Syringe		
17	3263	48 days	Lateral anastomosis	Died, pneumonia	No	Syringe		
18	3285	51 days	End-to-end anastomosis	Killed, chloroform	No	Syringe		
19	3286	53 days	End-to-end anastomosis	Killed, chloroform	No	Syringe		
20	3281B	60 days	Enterorrhaphy	Killed, chloroform	No	Syringe		
21	3328	64 days	Enterorrhaphy	Killed, chloroform	No	Syringe		
22	3325	144 days	Enterorrhaphy	Killed, ether	No	Syringe		

TABLE II

Exp. No.	Surg. Path. No.	Time Post-op.	Cause of Death	Leak or No	Mm. of Water Pressure	Lbs. per Square Inch	Type of Preliminary Operation
1	3521	1 hour	Killed, chloroform	No	700		Strangulation with tape.
2	3522	4 hours	Killed, chloroform	No	800		Strangulation with tape.
3	3436	1 day	Killed, chloroform	No	660		Paraffin in mesenteric vessels.
4	3452	1 day	Killed, chloroform	No	680		Strangulation with tape.
5	3468	1 day	Died peritonitis	Leak			Paraffin in mesenteric vessels.
6	3402	2 days	Died general peritonitis	Leak			Strangulation with tape.
7	3453	3 days	Killed, chloroform	No	720		Strangulation with tape.
8	3477	3 days	Killed, chloroform	No	720		Strangulation with tape.
9	3490	5 days	Died, general peritonitis	Leak			Strangulation with tape.
10	3570	7 days	Killed, chloroform	No	1400	2	Strangulation with tape.

The times given above refer to the time elapsing between the second operation and the death of the animal. The second operation in every case in this series was an end to end entero enterostomy. Twenty-four hours elapsed between the first and second operation in every case except experiment No. 8, when only six hours intervened. The pressure in every case was measured by the water manometer.

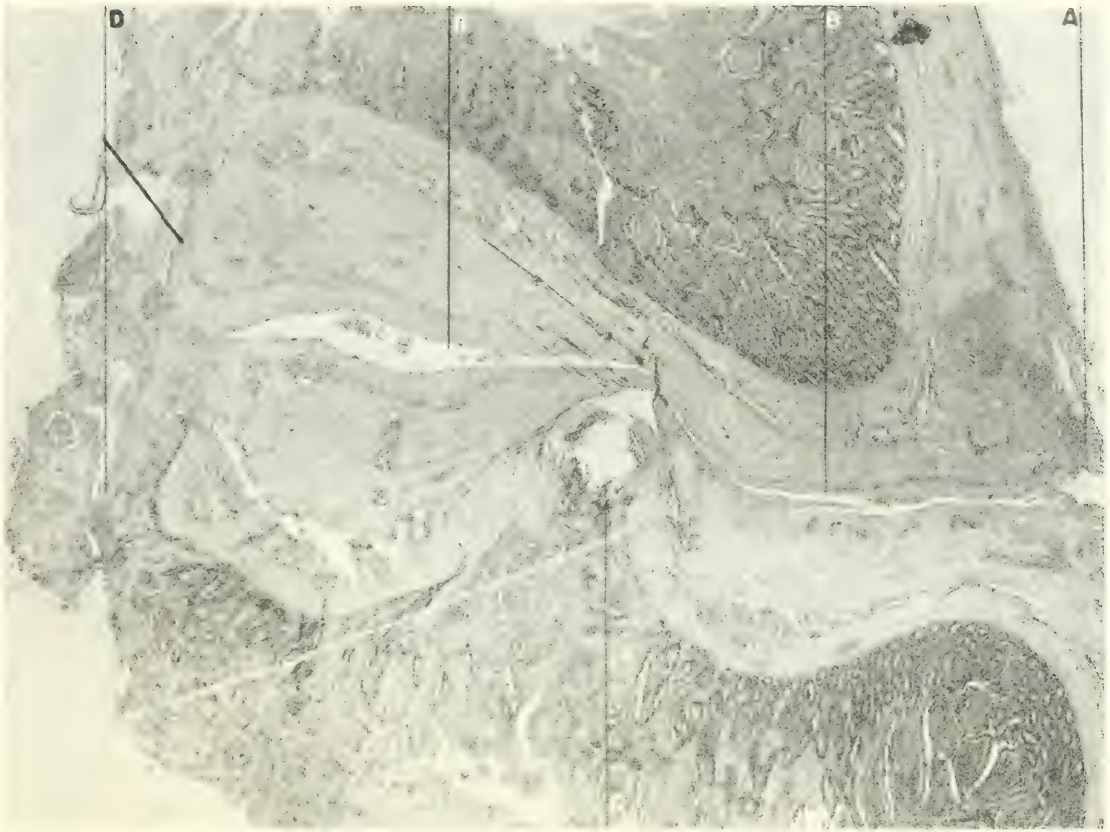

Fig. 2. Lateral entero-enterostomy one hour after operation, Surgical Pathology No. 3262D. *A* Coagulum filling space between approximated serous surfaces; *B* line of approximation of infolded serous surfaces; *C* silk suture passing through submucosa; *D* infolded cut edges of intestinal coats projecting into lumen.

exposed to a very high internal pressure, and, second, to the fact that the dogs have received no food for 12 hours previous to operation. This latter element is probably the more important since Magnus has shown that an animal recently fed shows very much better intestinal contractions than one that has been fasting.

In all the experiments the movements of the gut which are carried out in close proximity to the site of injury are invariably smaller than those made by the intestine 10 or 20 times more distant.

The interval elapsing between the time of operation of anastomosis and the graphic recording of the movements does not modify the strength of the contractions. Thus, in experiments which have been carried out in a dog killed one hour after anastomosis, the contractions are no larger than in experiments that have been made four days after a similar operation. A moderate amount of acute inflammation about the suture line does not interfere with the movements of the muscle, either near the suture or at a considerable distance from it; thus, in one experiment the contractions made by a ring of muscle 5 mm. from the suture were quite as well defined as those made 150 mm. from the suture line.

Only one experiment was carried out on an intestine after gangrene had been produced. In this particular instance, muscle 60 mm. from the suture line contracted well, whereas muscle 5 and 15 mm. from the suture line remained absolutely quiescent. This quiescence cephalad to the injured area would seem to be a protective mechanism. It has been shown that when gangrene occurs the suture line does not withstand an increase in internal pressure. Normal movements of the intestine are undoubtedly associated with an increase in internal pressure. And if weakness occurs at the line of suture, an escape of intestinal contents into the peritoneal cavity will occur. From these experiments, then it appears that the inhibition of the intestine cephalad to the

suture aids in preventing the escape of intestinal contents into the peritoneal cavity.

The movements which have been recorded are not true peristaltic movements. They represent only the segmenting movements of Cannon. No attempt has been made to record graphically true peristaltic contractions. Careful observation has never shown a peristaltic wave spread across the suture line.

The conclusion that may be drawn from these experiments is that, despite the very severe operation, the smooth muscle of the intestine retains its viability and its segmenting function. This function is demonstrable within one hour after the completion of the operation and is present four days after operation. In the presence of gangrene and a leaking suture, these segmenting movements are inhibited cephalad to the suture, whereas caudal thereto the movements are regular, powerful, and well-defined.

ANATOMICAL AND HISTOLOGICAL OBSERVATIONS ON THE PROCESSES OF REPAIR AND REGENERATION

From a gross and microscopic examination of the thirty-two specimens comprising the two series, together with an examination of many similar suture operations done in this laboratory, and a comparison of these observations with those of Mall, Gould, and others, a fairly comprehensive picture of repair and regeneration in healing intestinal wounds has been obtained.

In the course of an operation that divides and then sutures the coats of an intestine, a certain amount of damage is done. The knife that has severed the intestinal coats and the scissors that has trimmed off the redundant mucous membrane, have killed many cells and fatally injured many more. Handling the peritoneum has done some damage to its serous cells. The needle, while passing through the wall of the gut, injures some of the small blood-vessels. It may also tear off

the bottoms of some of the glands and crypts of the mucosa, and may even carry living epithelial cells along the suture tract deep into the submucosa or muscularis. Finally, slight contamination of the tissues in and about the wound is inevitable.

As a result of these injuries, when the suture operation is completed, the blood that has escaped on the free peritoneal surface and between the approximated infolded serous coats coagulates in a wedge-shaped mass that plugs the line of suture throughout its entire extent (Fig. 2, *A*). There is also a certain amount of coagulated blood in the substance of the intestinal wall and about each suture hole. This coagulated blood is, no doubt, of importance in preventing leakage due to possible faulty operative technique. That it is not essential in preventing leakage if no technical errors are made, would seem probable from the two anastomoses done on the intestine of a dead dog, in which no coagulation of blood took place and yet which did not leak when subjected to extremely high pressures.

Depending on the amount of trauma done to the peritoneum and upon the quantity of blood extravasated, more or less extensive fibrinous adhesions occur. These develop very rapidly between the line of suture and the surrounding serous surfaces, more particularly that of the omentum.

During the first hour following suture, polymorphonuclear leucocytes make their appearance in the region of the wound. They may be seen in and about the engorged blood-vessels, invading the coagula and collecting about the buried sutures. Exudation of serum has begun by the end of the first hour. It is first noticeable between the muscle bundles of the muscularis.

Degenerative changes in those tissues which are fatally injured by the operative trauma no doubt start at once, for it can be seen microscopically, along the cut mucosa edges, four hours after operation.

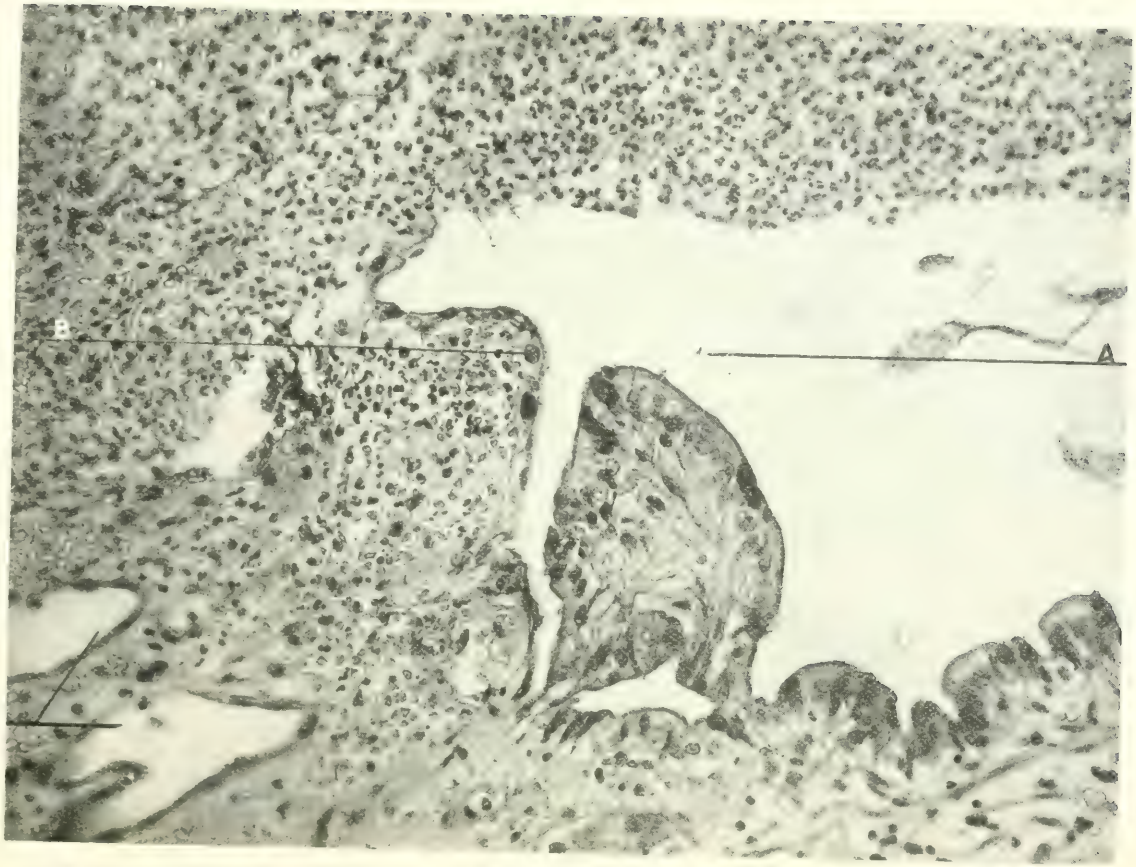

Fig. 3. End-to-end entero-enterostomy 24 hours, Surgical Pathology No. 3312. *A* Sulcus between living mucosa and infolded cut edges of intestinal coat; *B* newly formed epithelial line.

Reparative processes also no doubt begin very quickly. The infolded cut edges of the intestinal wall make a ridge which juts into the lumen of the intestine (Fig. 2). The summit of this is composed of the exposed cut edges of the intestinal coats. Somewhere on the sloping sides of this ridge are the junctions between the living mucosæ and the denuded area. This junction is often marked by a sulcus (Fig. 4, *A*). Within 24 hours fibroblasts can be observed proliferating just beneath the necrotic tissue and fibrin, which cover the denuded area. Fibroblasts can also be seen beginning to invade the edges of the blood coagula in other parts of the specimen. In the sulcus at the edges of the ridge, the epithelium lining the adjacent intact mucosa starts to proliferate, growing in a flattened syncytial layer over the denuded surface. The appearance of this new forming epithelium closely resembles the embryonal form (Fig. 3, *B*). The cystic downgrowth of newly formed glands into the submucosa has begun by the end of 24 hours. This was described by Mall but he did not observe it until the sixth day.

During the next three days the congestion, the œdema and the leucocytosis are on the increase. Grossly, the fibrinous adhesions become progressively firmer and when torn away leave a reddened suture line with swollen edges. Inside the lumen, the degenerated and dead tissue is separated from the living and is cast off. The denuded surface of the ridge becomes smoothed over with a thin layer of fibrin, beneath which, granulations are springing up. From the sulci on either side, the epithelium is growing over this smooth surface beneath the fibrin.

In seven to ten days the coagulum disappears and is replaced by new connective tissue. This contracts so that it is difficult to tell, microscopically, exactly where the line of approximation is. The time required to cover the exposed surface of the inverted ridge is extremely variable. It depends largely

Fig. 4. End-to-end entero-enterostomy, Surgical Pathology No. 3282. *A*, Showing sulcus between viable mucosa and denuded area, *B*, denuded area covered with granulation tissue and fibrin.

on its surface measurements and elevation. Mall found it almost covered with a new mucosa after 15 days. In one of our cases it was not completely covered after 38 days. The average is about 23 days. The formation of new glands proceeds almost as rapidly as the growth of the new layer of the surface epithelium. The newly formed mucosa is at first thinner than normal and has no muscularis mucosæ. After two months have passed, however, it is completely regenerated and cannot be distinguished from the surrounding undamaged mucosa.

After the seventh day the fibrinous adhesions between the suture line and the surrounding serous surfaces have, for the most part, disappeared. Those that remain, become organized, so that by the fourteenth day they are dense and fibrous, and can only be torn away with great difficulty. As suggested by Prof. W. C. Clarke, most of these also disappear gradually as the months pass; the movements that go on constantly within the abdominal cavity by gentle traction probably cause stretching of the connective tissue fibrillæ, disappearance of the blood-vessels, and gradual atrophy and disappearance of the adhesion band. It is common to find after one month only a single narrow band running from the cicatrix in the gut to the omentum or mesentery.

The realignment of the intestinal wall which is the fourth and final stage of repair according to Mall, was not as complete in our cases as he seemed to find it. In none of the specimens examined by us did the infolded ridge disappear entirely. It undoubtedly grows much smaller. The circular muscle appears to be realigned, but we were always able to see connective tissue interrupting the course of the muscle-bundles. Marchand (10) said in this connection: "The regeneration of the muscle-bundles is not abundant enough to bridge small spaces of a few millimeters — if, however, muscle wound lies against muscle wound, the scar macroscopic

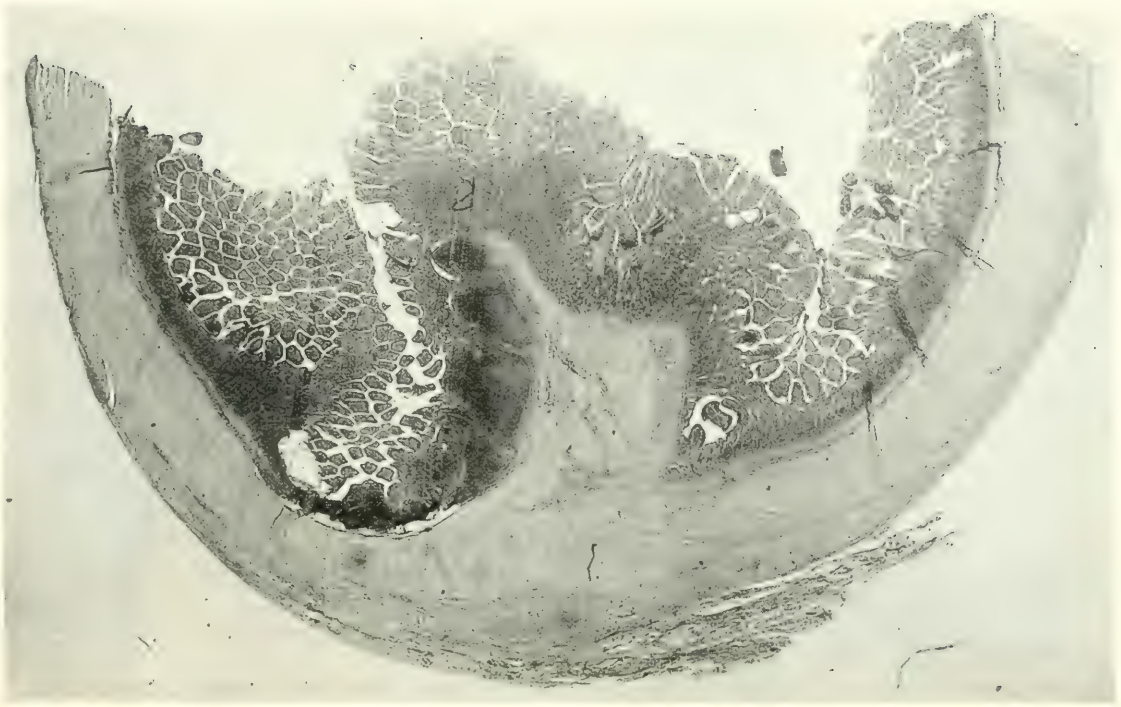

Fig. 5. Enterorrhaphy sixty days after operation (Surgical Pathology No. 3282) showing complete regeneration of mucous membrane and realignment of muscular coat.

ally would conceal itself in the unbroken line of the fibers." That a certain amount of regeneration of smooth muscle can occur seems certain.

The epithelial lined cysts and tubes with adenomatous growths about them deep in the wall of the gut, which were first observed by Mall, we found in four of our cases, ranging from 51 to 144 days after operation. They occur usually about a buried suture and have many polymorphonuclear leucocytes in and about them. Mall believed that they were implanted by the needle at the time of operation. They appear to be of purely pathological interest and to have no surgical significance.

Some remnants of the silk sutures were found in every case. In the older ones there were usually one or more loose ends lying free in the lumen, covered with gritty material.

SUMMARY AND CONCLUSIONS

The following conclusions based on the data obtained from the operations on the normal and the gangrenous small intestine of the dog, are grouped together for the reason that in both series the experiments were the same and the end-results identical.

1. The non-infected suture line in the small intestine in dogs is very resistant to internal hydrostatic pressure. For at one hour after operation and any time thereafter the area of operation is capable of withstanding an hydrostatic pressure of over one pound per square inch without leakage.

2. The clinically infected specimens leaked at minimum pressures.

3. To obtain perfect results a proper technique is essential. For it is seen that in a dog recently killed the intestine, when properly sutured, is capable of withstanding a pressure of nearly two pounds per square inch without leakage.

4. Imperfect technique results in a defective suture line. The defects, if not too ex-

tensive, may be sealed by the coagulum which probably prevents leakage.

5. The smooth muscle of the divided and sutured intestine retains its viability and segmenting function to within 5 mm. of the line of suture.

6. In an infected case with gangrene around the suture line, no segmentation occurred within 15 mm., while 60 mm. away contractions were powerful and well defined.

7. Repair in sutured intestinal wounds begins at once with the coagulation of the extravasated blood which fills in the space between the two approximated serous surfaces. This union becomes permanent in from 7 to 10 days, with the replacement of the coagulum by connective tissue.

8. Repair of the mucosa is first seen after 24 hours beginning with a line of syncytial epithelial cells extending from the edge of the viable mucosa over the denuded surface of the infolded cut edges of the intestinal coat.

9. The denuded surface may be covered with an immature mucosa as early as the fifteenth day (Mall), but it is usually not completely covered until 23 days after operation.

10. Regeneration of the mucosa is complete after two months.

11. Complete anatomical regeneration of the muscularis does not occur. A realignment of the infolded muscular fibers occurs, but it is always interrupted by a thin line of scar-tissue.

From the above data we conclude that fluid and food may be given immediately after operation without danger of leakage in the sutured small intestine. If leakage does occur, it is due to infection or faulty operative technique.

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